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Fate and Effect of Oil in the Aquatic Environment

Gulf Coast Region

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July 1980

FATE AND EFFECT OF OIL IN THE AQUATIC ENVIRONMENT - GULF COAST REGION

by

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Contract No. 68-01-0745

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FOREWORD

The Environmental Research Laboratory of the U.S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a great extent, this requires research on the tolerance of marine organisms and their life stages as well as of ecosystems to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

This report describes a multidiciplinary study of the distribution and alterations of crude oil in marine systems of the coastal Gulf of Mexico. Biological uptake of and response to spilled, mechanically dispersed, and absorbed oil was followed. Studies include both laboratory and field systems used to assess chronic effects and ecosystem recovery.

Tudor T. Davies

Director

ABSTRACT

The purpose of this research investigation was to determine the fate and effect of crude oil in the aquatic environment of the coastal Gulf of Mexico. The project was multi-disciplinary and multi-institutional in scope and involved both laboratory and field sized pilot-plant ecosystem studies. Emphasis was placed on the long-term, low-level chronic effects of oil pollution on the ecosystem. Of the five crudes employed in the investigation, Empire Mix crude was studied most intensively.

The following conclusions were drawn from the investigation.

After an oil spill, the oil only remains in the water column for a short period of time (days) and migrates into the sediments where it persists for long periods of time (years). Surface oil which migrates into the marshes will reduce marsh plant productivity by up to 88% and will reduce the rate of conversion of the grass into utilizable detritus by up to 50%. Oil in the marsh grass systems serves as a mechanism for reentry of oil into the estuarine food web.

Oil, even at levels which would result in a slick only 0.01 mm in thickness, drastically reduces the zooplankton population (particularly Acartia) but recovery of the population to pre-spill levels occurs within 10-15 days. The major initial impact of a spill of this magnitude on higher members of the food chain (shrimp and mullet) is an alteration in behavior which will make them susceptible to predation for the period of time they are exposed to the oil. Physiological changes (as evidenced by enzymological and fatty acid analyses) in these organisms are evident and appear to be a stress response.

Long-term chronic effects of oil on the ecosystem probably will occur but the severity of the impact will vary from oil to oil and the environmental conditions. Uptake of oil immediately after a spill or constant exposure to low levels of oil in the environment (sediments and detritus) has a debilitating effect on the higher elements of the ecosystem which may manifest itself in one or more of the following ways: increased disease (fin rot), histological changes and decreased growth in fish; increased disease in shrimp; and decreased growth and increased mortality in oysters.

This report was submitted in fulfillment of Contract No. 68-01-0745 by Mississippi State University under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers a period from June 28, 1972, to February 29, 1976, and the work was completed as of April 15, 1976. The University of Southern Mississippi and the Gulf Coast Research Laboratory served as subcontractors to Mississippi State University on this Contract.

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SECTION I

INTRODUCTION

The cause and effect relationship between oil pollution and its acute effects on the aquatic ecosystem is easily established because of the brief time span between the impact of the oil and the observed effect. To the contrary, the chronic effects of oil on the ecosystem are more difficult to establish, because the onset of observable effects may require months or years.

Most reports on the effects of oil pollution involve either studies conducted in the environment after major oil spills or laboratory studies on individual members of the ecosystem. Both of these types of studies have shortcomings in terms of estimating the true impact of oil on the aquatic environment. In the first case, lack of adequate pre-spill data, use of other chemicals in clean-up procedures, inability to obtain representative samples, etc., seriously limit the value of data regarding identification of chronic effects or establishing the effects of low-level oil pollution. In laboratory studies there is always the question of the validity of extrapolating the results to the natural environment because of the complexities of the system.

The use of a pilot-plant ecosystem seems to be one of the ways of obtaining meaningful data on the chronic effects of low-level oil pollution. It affords an opportunity to verify laboratory data on a manageable field scale, and the results should be more applicable to the natural environment.

It is obvious that a study involving the total biota of the ecosystem would be economically impossible and scientifically unmanageable. Similarly, since crude oils vary widely in composition, extensive testing with all types of oil would be economically impractical. Therefore, certain decisions had to be made at the outset in regard to which crude oil should be employed and which members of the ecosystem would be investigated. As a result of these considerations, the following decisions regarding the proposed study were made at the outset. A majority of this investigation was performed with Empire Mix crude oil, since it typifies the oil produced in the Gulf Coastal area and thus would be typical of the oil spilled during a drilling accident (the most prevalent type of oil spill).

The salt marsh estuarine ecosystem is essentially a detritus-based system. Of the three groups of primary producers in the system, the bulk of the organic matter biomass is contributed by marsh plants. Phytoplankton and diatoms also are important primary producers and thus were included in this investigation. Since zooplankters represent the first link in the food chain above the primary producers, they likewise were investigated in the study.

Mullet are considered to be omnivorous filter feeders but, to a large extent, are herbivorous and were included since they might readily reflect changes in the lower members of the food chain.

The economically important oyster was selected as a representative of sessile, inshore benthic animals.

Shrimp are one of the most valuable fisheries resource in the Gulf Coast area, and their food habits and dependence upon the estuaries make them an excellent choice for detecting subtle changes in the estuarine environment.

This report is a summary of the results of a three and one-half year study of the fate and effect of oil on the aquatic environment of the Gulf Coast Region. Phase I involved laboratory studies on selected members of the ecosystem and the establishment of an estuarine pilot-plant ecosystem. Phase II consisted of (1) following the results of a heavy spill using

Empire Mix crude oil in a naturally-occuring estuarine tidal pond, (2) monitoring the results of a low-level spill using Empire Mix crude oil in the estuarine pilot-plant ecosystem for a period of eleven months under conditions simulating tidal action and (3) following the effects of three different crude oils (Empire Mix, Saudi Arabian, and Nigerian) on separate estuarine pilot-plant ecosystems over a period of nine months in the absence of tidal action.

All graphic and tabular data (Appendix) are available in hard copy or microfiche from NTIS only, National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.

SECTION II

CONCLUSIONS

The use of a tidal-simulation pond system as a mini-ecosystem seems eminently sound as a complement to laboratory studies. The system afforded an opportunity to study a partially reconstructed ecosystem under conditions more closely resembling the natural environment than those achieved in the laboratory and yet the system is manageable while the natural environment is not.

Based upon two pilot-plant ecosystem studies, the following conclusions were drawn in regard to the fate and effect of oil in the aquatic environment of the Gulf Coast region.

After an oil spill, the oil remains in the water column for a short period of time (days) and then migrates into the sediments where it persists for long periods of time (years). Surface oil which migrates into the marshes will reduce marsh plant productivity by up to 88% and will reduce the rate of conversion of the grass into utilizable detritus by up to 50%. Oil in the marsh grass systems serves as a mechanism for reentry of oil into the estuarine food web.

Oil, even at levels which would result in a film only 0.01 mm in thickness, drastically reduces the zooplankton population (particularly Acartia) but recovery of the population to pre-spill levels occurs within 10-15 days. The major initial impact of a spill of this magnitude on finfish and crustacea is an alteration in behavior which will make them susceptible to predation for the period of time they are exposed to the oil. Physiological changes (as evidenced by enzymological and fatty acid analyses) in these organisms are evident and appear to be a stress response.

Long-term chronic effects of crude oil on the estuarine ecosystem would be expected to occur on the basis of our investigation. The severity of the impact would vary with the specific oil employed and the environmental conditions existing at the time. Uptake of oil immediately after a spill or constant exposure to low levels of oil in the environment (sediments and detritus) was found to have a debilitating effect on the fish, shellfish and crustacea which manifested itself in one or more of the following ways: increased disease (fin rot), histological changes and decreased growth in fish; increased disease in shrimp, and decreased growth and increased mortality in oysters.

SECTION III

RECOMMENDATIONS

The results obtained in this investigation clearly indicate the desirability of conducting some additional pilot-plant ecosystem studies directed toward answering certain key questions regarding the effect of oil pollution on the estuarine ecosystem. The studies should be mission-oriented in that the information obtained would be utilizeable by management in making decisions on such matters as (1) selecting the clean-up method for oil spills which would have the least damaging effect on the ecosystem, (2) determining if and what environmental reclammation procedures could be employed in polluted areas and (3) more accurately assessing damages in oil spill cases.

One study should be directed toward determining whether the chronic effects of oil on shrimp, mullet and oysters were the result of the initial exposure to the oil or the constant exposure to oil and oil degradation products in the environment (sediment and detritus). One experiment would be designed to subject specimens to low levels (as employed in the present investigation) of oil for two to four days and periodically monitoring them in a clean environment for a period of one to two years. In a companion experiment, the system should be subjected to the same level of oil for seven to fourteen days prior to the introduction of the test shrimp, mullet and oysters.

A second pilot-plant ecosystem study should be conducted in a system expanded to include one or more predator fish (e.g. speckled trout), one or more scavengers (e.g., crabs) and an increased quantity of marsh grasses. These studies should extend over at least two years and should include a comparison between (1) oil allowed to remain on the surface as long as possible and (2) oil entrained into the water column through mechanical means (*e.g.*, increased turbulence).

Some additional laboratory studies would also seem to be justified. Specifically, the following areas should be addressed: (1) the mixed function oxidases in mullet liver as they relate to oil metabolism, (2) the role of oil in fin rot disease in fish and (3) white eye syndrome in shrimp.

SECTION IV

MATERIALS AND METHODS

MATERIALS

Crude Oils

The Empire Mix crude oil employed for a majority of the work performed under this contract was kindly supplied by the Standard Oil Co., Pascagoula Refinery, Pascagoula, Mississippi. Representative analyses of the oil also were supplied by the Refinery. [1]*

The other crude oils employed in this investigation were kindly supplied by Exxon Co. from the following sources: Saudi Arabian crude oil from the Baytown, Texas, Refinery; Venezuelan (La Rosa) crude oil and Nigerian (LT) crude oil from the Exxon Refinery at Bayonne, New Jersey; and the Iranian (heavy crude) crude oil from the Exxon Refinery at Benicia, California.

Collection and Treatment of Biological Specimens

Mullet, Shrimp and Oysters-

All specimens of mullet (*Mugil cephalus*), shrimp (*Penaeus aztecus*) and oysters (*Crassostrea virginica*) were collected from the Mississippi Gulf

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*Numbers in brackets at the end of a paragraph refer to items in Appendix

Coast area. For laboratory studies, the organisms were held for at least 7 cays before being employed in bioassays.

Phytoplankton-

Phytoplankton were collected from the Mississippi Sound in a 200 mesh plankton net (#25 Turtox), using a boat speed of 5 knots or less and a towing time of 3 to 5 min. An aliquot, preserved in 10% formalin, was counted under standard procedures (strip-count method) for both numbers and species diversity.

Living phytoplankton samples were refrigerated, returned to the laboratory and placed in enrichment media (Heinle or N.H.). Single cells of representative organisms were obtained from the enrichment cultures, washed in sterile sea water and placed in test tubes of Heinle or the N.H. medium. These cultures were grown and maintained as stock cultures and transferred every 12 days.

As a rule the salinity in the collection areas range from 20-28 o/oo.

Zooplankton-

Zooplankton samples were collected in a 125 mesh net (#20 Turtox), using a boat speed of 5 knots or less and a tow time of 2 min or less. Samples were preserved in 10% formalin and counted by placing concentrated samples in a petri dish and observing them with a stereo microscope.

Living samples were maintained at the collection temperature in 19-liter oxygenated plastic sacks, placed in a styrofoam ice chest and transported to the laboratory. Cultures were maintained in 20-40 liter aquaria at ambient temperature. Individuals were withdrawn from the aquaria with glass tubes (at least 6 mm I.D.) and subjected to a variety of conditions for growth and maintenance.

Marsh Plants-

All marsh plants were collected from the Mississippi Coastal areas (particularly the Bay St. Louis area and the Gulf Coast Research Laboratory area). Plants for laboratory analyses were clipped at the base about 5 cm from the surface of the mud, thoroughly cleaned of debris and silt and air dried. Plants for transplanting were dug in clumps of about 60 cm diameter and 60-90 cm deep to collect the rhizomes and roots.

ANALYTICAL METHODS

Oil in Water by Infrared Analysis

Five g of NaCl, 2.5 ml of 1:1 (v/v) sulfuric acid, and 25 ml CCl_4 were added to 1 liter of water in a separatory funnel. The sample was shaken vigorously for 1 min, the phases allowed to separate and the CCl_4 extract passed through a column containing a 2.54 cm layer of anhydrous Na_2SO_4 (CCl_4 washed). This procedure was repeated four times and the extracts combined. Oil was measured at an absorption band of 2930 cm^{-1} using a 10 mm path length cell and compared to a standard CCl_4 solution containing a known amount of oil.

Oil in Water by Gas Chromatography

An 800 ml sample of water was placed in a 1 liter separatory funnel and extracted 3 times with 100 ml of hexane and treated as described by Miles et al. (1975). Analyses were conducted using a Beckman GC-45 gas chromatograph with a flame ionization detector with a 183 cm x 0.32 cm OD stainless steel column packed with 3% SE-30 on 80-100 mesh Chromasorb W. The injector and detector temperatures were 300 C and the column temperature was programmed from 100 to 300 C at a rate of 3 C per min. Qualitative identification of the components was achieved by comparison to retention times of known standards. The quantitative measurement of the total oil in the sample was calculated from the area of the $\underline{n}$-C_{16} peak.

Oil in Sediments by Gas Chromatography

The gas chromatographic procedure for determining oil in sediments was carried out in accord with the method described by Lytle (1975).

Oil in Sediments by Liquid Chromatography

The sediment sample (100 g wet wt) was soaked in 100 ml of MeOH (for heavy mud samples) or in 100 ml of hexane and MeOH (90 ml:10 ml) (for samples composed largely of shells or rocks). The sample was filtered through a Buchner funnel containing Whatman #43 filter paper, washed with 100 ml of hexane and both extracts combined and evaporated in vacuo to approximately 1 ml. This concentrated extract was transferred to a small vial, evaporated under nitrogen, dissolved in 10 μl of hexane and analyzed using a Waters Associates Model 202/401 liquid chromatograph with a UV detector (wavelength, 277 nm). A 2.54 x 0.63 cm OD micro Bondapak C_{18}/corasil column was employed with a $MeOH:H_2O$ (70 ml:30 ml) solvent system at a flow rate of 2.0 ml/min.

Qualitative identification of the components was achieved by comparing the retention times of oil components with known standards.

Estimation of total oil was made by integrating the total area for all peaks obtained by analysis of the aromatic hydrocarbon fraction from the control and test organisms. Integration was performed with a 3388 Hewlett-Packard automatic integrator. Corrections for control absorbance were made. Results were calculated as μg oil by comparison to standards.

Oil in Mullet, Shrimp and Oysters

The samples were homogenized in TenBroeck Tissue grinders at 10% (w/v) in 0.2 M sodium phosphate and 0.25 M sucrose. For every gram of tissue, 10 ml of 0.75 M KOH in 2% aqueous methanol solution was added, and the resulting mixture was refluxed for 15 hrs. The saponified samples were extracted 4 times with hexane (the amount of hexane used for each extraction was 1/4 to 1/3 the amount of the volume of total saponified solution). The hexane extract was washed with water, dried with sodium sulfate and evaporated in vacuo. The material was washed onto a chromatographic column (with a 70-100-µ-fritted disc packed with 4.5 g 60-200 mesh Activity I silica gel and topped with 2.5 g 80-200 mesh Activity I neutral alumina) with 5 ml of hexane and eluted with 75 ml of hexane followed by 85 ml of benzene. Each fraction was evaporated in vacuo to 1 ml and transferred to a small vial, where the remaining solvent was removed under nitrogen. The hexane eluate was employed for analyses of aliphatic compounds as described in the section on Oil in Water by Gas Chromatography. The benzene fraction was employed for analyses of aromatic compounds as described by Miles et al., (1975) or as described in the section on Oil in Sediments by Liquid Chromatography.

Oil in Marsh Plants

The marsh grass samples were pre-washed with soap and water and wiped with a hexane-soaked cloth. Five grams of the finely ground material were soaked over night in 50 ml of hexane, filtered and evaporated in vacuo.

The samples were then treated in the same manner as the animal samples (beginning with the addition of KOH).

Determination of Dissolved Oxygen

Both the Azide Modification of the Winkler Method ("Standard Methods for the Examination of Water and Wastewater", 13th Ed.) and a YSI Model 54 Oxygen Meter were employed for determining dissolved oxygen.

Fatty Acids in Mullet, Shrimp and Oysters

After saponification of the tissue as described in the section on Oil in Mullet, Shrimp and Oysters, the water layer containing the fatty acids was acidified with 6 N HCl to about pH 3. The water layer was extracted three times with 30 ml $CHCl_3$ and the extract rotovaped to dryness. The sides of the flask were rinsed with a small amount of MeOH and diazomethane added until the reaction was complete, then rotovaped to dryness in a roundbottom flask. Thirty mls of water were added to the flask, the contents transferred to a separatory funnel and the water layer extracted 4 times with 20-30 mls of hexane each time. Each extraction was drained through Na_2SO_4 into another roundbottom flask and rotovaped to 1-2 mls volume. The extracted fatty acid methyl esters (FAME) were run through 8-18 cm of silica gel column with 45-85 mls of benzene and collected in a flask. The benzene was rotovaped down to 1 ml and transferred to small weighed vials. The sides of the flask were washed down with ether and added to the sample in the vials. The solvent was evaporated under nitrogen leaving the purified FAME. The sample was placed in a desiccator for at least 1 hr and reweighed. The purified FAME were stored under nitrogen in the refrigerator until analyzed by gas chromatography.

The purified samples were diluted with pesticide grade hexane, the ratio being 1 mg sample to 10 ml solvent. Approximately 1 μl was injected through the rubber septum into the vaporization chamber of the inlet with a Hamilton 701 RN syringe. The solvent flush technique of Kruppa (1971) proved to be the most successful method of sample introduction.

All FAME were analyzed with a MT-220 GLC equipped with a hydrogen flame ionization detector. The columns used were 3.17 cm by 1.83 m aluminum or stainless steel packed with 15-20% diethylene glycol succinate (DEGS) supported on 80/100 mesh chromasorb WAW. Operating conditions were as follows: Carrier flow (N_2), 22 cc/min; Column temperature, 190 C; Detector temperature, 225 C; Inlet temperature, 200 C; Hydrogen flow rate, 50 cc/min; and Air flow rate, 34 liters/min.

Qualitative Identification of Peaks-

Purified FAME standards from the Hormel Institute were compared with the retention times of the FAME for identification of individual peaks on the chromatogram. The fatty acid methyl ester standards were: 14:0, 15:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3, 19:0, 20:0, 20:4, 20:5 and 22:6.

Identification of some unsaturated and saturated fatty acids was accomplished by plotting the log of the retention times versus the number of carbons in the chain. This procedure allowed the tentative establishment of the number of carbons and the number of double bonds for the fatty acids contributing peaks to the chromatographic record.

Further identification of the unsaturated fatty acids was obtained by hydrogenation. Hydrogenation converted the unsaturated acids to saturated acids, and thus the chain length of the various acids was confirmed. Hydrogenation of the unsaturated fatty acids was accomplished using Adam's platinum oxide catalyst. "Spiking" of the sample served to identify positively such fatty acids as 14:0 and 18:3.

The method of Carroll (1961) was used for the quantitative estimation of peak areas in the fatty acid spectrum. This method involves the multiplication of the peak height by the retention time of each fatty acid. The area under the peak represents the relative amount of that component in the mixture.

Histological Examination

Immediately upon collection, specimens were placed in 10% formalin solution for preservation.

Shrimp, mullet and oyster tissues were fixed in either buffered neutral formalin or Bouin's or Davidson's fixatives. After a minimum of 24 hrs in fixative, the tissues were processed in an Autotechnicon Mono® tissue processor and embedded in paraffin. Four to six micron thick sections were then stained with hematoxylin eosin for routine histological study.

Microbiological Methods

Routine Counting Procedures-

Media and methods employed in the microbiological monitoring programs were as follows. All plate counts were conducted using the conventional spread plate technique and media containing 17.13 g/l Rila Marine Mix. Total bacterial counts were made using nutrient agar (2% agar, w/v) with incubation for 2 and 7 days. Total yeast counts were made using potato dextrose agar (2%, pH = 3.5) with incubation for 5 days. Total fungal counts were made using Cooke's Rose Bengal Agar (pH 7.0) with incubation for 5 days (observations for actinomycetes were made after 10 days).

Estimates of hydrocarbon-utilizing, nitrate-reducing microorganisms and hydrocarbon-utilizing, sulfate-reducing microorganisms were conducted using a modification of the method described by Rosenfeld (1960) wherein Empire Mix crude oil was employed as the carbon source and the 3-tube MPN technique employed. Incubation was for 10 and 21 days.

Microbiological Degradation of Oil-

These tests were conducted in 14-liter fermentor jars containing 10 liters of the following medium: 40.00 g of $(NH_4)_2SO_4$, 5.06 g of KH_2PO_4, 14.94 g of K_2HPO_4, 171.30 g of Rila Salt mix and 10 liters of distilled water.

After solubilization of the salts, the medium was filtered to remove insoluble precipitates. Fifty grams of Empire Mix crude oil was then added and incubation carried out at room temperature on either a Microferm Laboratory Fermentor (Model No. MF-114) or a Model No. FS-614 (both from New Brunswick Scientific Co). Agitation was set at 200 rpm's and aeration at 10 psi of air.

Ten grams of mud (from the Bay St. Louis area of Mississippi) and 300 ml physiological saline were blended at low speed for three minutes, allowed to settle and 100 ml of supernatant withdrawn for use as an inoculum.

Samples were withdrawn as desired for analyses.

Bioassay Procedures

Preparation of Oil-

The crude oil was emulsified using an ultrasonic dismembrator at 70 watts for 3 min. The oil-water emulsion was then introduced into the test aquaria.

Mullet, Shrimp and Oysters in 114-liter Aquaria-

All glass aquaria (114 liters) were filled with artificial sea water (Rila Mix), salinity of 15 o/oo. Aeration was accomplished using a subsurface Dynaflow® underwater filtering system in which there was continuing aeration through the Dynaflow® filter. The temperature was held constant at 20 C. Water samples were taken routinely for dissolved oxygen and oil determination.

In general 6-8 animals were placed in each aquarium and the emulsified oil preparation mixed into the test aquarium. Animals were placed in the aquaria 24 hr before testing.

Mullet, Shrimp and Oysters in 1,895-liter Tanks-

Many of the laboratory tests concerned with the effects of low levels of oil over a prolonged period of time were conducted in 1,895-liter tanks. A 1,895-liter tank was filled with artificial sea water (15 o/oo) and two pumps used for circulation and filtration of the water. The pump circulated the water at 0.7 revolution/min or 947.5 liters/hr. Water samples were taken routinely for dissolved oxygen and oil determinations.

Phytoplankton-

Both Heinle medium and N.H. medium were employed routinely. Heinle medium has been found to be superior for those organisms requiring salinities of less than 20 o/oo while N.H. medium was employed for the higher salinities. Standard growth conditions for toxicity tests consisted of a 12-12 hr light-dark cycle, 20 C, pH 8.0-8.4 and salinities of 20-28 o/oo. Culture vessels were 16 by 150 mm test tubes or 125 ml Erlenmeyer flasks for tests involving emulsified oil and 125 ml Erlenmeyer flasks and aquaria for tests employing oily sediments. Appropriate dilutions of emulsified oil in the medium were dispensed in 10 ml amounts in the test tube and 25 ml amounts in the flasks, inoculated and incubated. Sterility, if desired, was effected by autoclaving. For tests with oily sediments, enough of the oily sediments were added to the system to saturate the aqueous phase. Cell counts were made employing 1 ml aliquots and a Sedgewich-Rafter counting cell (strip-count method).

Zooplankton-

Five copepods were obtained from an aquarium (after acclimation) and placed in 50 ml of filtered natural aerated sea water containing various dilutions of the oil. Standard growth conditions for toxicity tests consisted of a 12-12 hour light-dark cycle and a temperature of 20 C. Usually the pH of the medium was in the range of 8.0-8.4 and the salinity in the range of 15-28 o/oo. The culture vessels employed were 150 ml beakers. Observations for mortality and natality were made using either a hand lens or a stereo microscope.

Other Methods

Mass Culture Techniques for Phytoplankton-

Heinle medium and/or N.H. medium were employed routinely. Standard growth conditions consisted of a 12-12 hour light-dark cycle, 20 C, pH 8.0-8.4, 20-28 o/oo salinity and a light intensity of approximately 2152 meter candles. The culture vessels were 4 or 9 liter inverted serum bottles aerated with filtered air. Length of incubation varied with the culture and ranged from approximately 4 days to 12 days. After incubation, 3.25 liters of the culture were withdrawn for use as a copepod food or for chemical analyses. Three liters of sterile medium were added to the culture and incubation repeated. If contamination were found, the culture was discarded.

Core Sample Culturing Techniques-

Cores were taken from all four ponds from the same area of each pond at two week intervals. Because the depth of each sample was difficult to regulate, 17 g of material were removed 8 cm from the top (soil-water interface) rather than from the "top" and "bottom" of the sample. Soil (17 g) was mixed with 20 ml BBM and agitated. Two ml of the mixture was placed on an agar plate and spread with a sterile glass rod. BBM agar and Heinle's agar at salinities of 3 o/oo and 20 o/oo were employed. All plating was done in duplicate or triplicate. Two ml of the mixture was added to 75 ml BBM liquid culture and 75 ml Heinle's media @ 2 o/oo and cultured for two wks. The colonies developing on the plates were counted and identified. The algae in the liquid culture were identified.

Enzymological Analyses

Preparation of Samples-

Brain, gill, liver and muscle of mullet and hepatopancreas and muscle of shrimp were homogenized in TenBroeck tissue grinders at 10% (w/v) in 0.1 M sodium phosphate + 0.32 M sucrose buffer (pH 7.6). Homogenate was centrifuged at 800 x g for 10 min at 0 C. The pellet was discarded. The supernatant of this centrifugation was designated as the "homogenate fraction". The supernatant of this centrifugation was spun at 8,000 x g for 15 min at 0 C. The pellet was the "mitochondrial fraction" and was resuspended to make a 10% equivalent. The supernatant was saved and was subsequently referred to as "supernatant fraction". The above centrifugations were performed on an International Refrigerated Centrifuge, Model B20A with a type 873 rotor.

For preparation of sample for NADPH-cytochrome c reductase assay, the above supernatant fraction was centrifuged at 110,000 x g for 60 min at 0 C. The pellet was suspended in 5 ml of 0.15 M KCl and centrifuged at 110,000 x g for 30 min at 0 C. The pellet was resuspended in 0.05 M potassium phosphate buffer in 10^{-4} M EDTA (pH 7.7) at a 40% equivalent. This was the "microsomal fraction". These centrifugations were performed in a Beckman Ultracentrifuge, Model L2-50, Type 50 rotor.

Oysters were removed from the shells, blotted dry and the whole organism was used. The oyster was ground initially in a Sorvall Omni-Mixer at 50% (w/v) in 0.1 M sodium phosphate + 0.32 M sucrose buffer (pH 7.6). This mixture was then homogenized in a TenBroeck tissue grinder. A portion of this homogenate was diluted to 10% with the same buffer and subjected to the centrifugation procedures described above. Another portion of the homogenate was diluted to 25% with the same buffer and sonified at 35 watts for 0.5 min with a Sonifier® Cell Disruptor, Model W 185, micro-tip (Heat

Systems - Ultrasonics, Inc.). This homogenate was then centrifuged at 110,000 x g for 30 min in a Beckman Ultracentrifuge, Model L2-50, Type 50 rotor, at 0 C. The supernatant was used and was referred to as the "sonified soluble fraction."

For microsomal mixed function oxidases in mullet liver, the liver was excised and homogenized in a TenBroeck tissue grinder in cold 0.15 M KCl. Homogenates were centrifuged at 9000 x g for 15 min in an International Refrigerated Centrifuge, Model B20A with a type 873 rotor. The supernatant was then centrifuged at 110,000 x g for 60 min in a Beckman Ultracentrifuge, Model L2-50, type 50 rotor. The pellet was resuspended in 0.15 M KCl and again centrifuged at 110,000 x g for 60 min. The pellet was resuspended at 500 mg equivalents/ml 0.15 M KCl. This microsomal suspension was used in all subsequent microsomal oxidase assays.

Enzyme Analyses-

All enzyme specific activities were expressed as mU/mg protein, where 1 mU is defined as 1 millimicromole of product formed/min. All cytochrome levels were calculated as nmoles/g. Protein concentrations were determined using the Folin-phenol reagent (Lowry, *et al*., 1951).

Lactate Dehydrogenase--(L-lactate: NAD oxidoreductase; 1.1.1.27). The procedure followed Boehringer Mannheim kit 15948 and Wroblewski and LaDue (1965).

Malate Dehydrogenase--(L-malate: NAD oxidoreductase; 1.1.1.37). The procedure followed Boehringer Mannheim kit 15981 and Bergmeyer and Bernt (1965).

Cytochrome Oxidase--(cytochrome *c*: O_2 oxidoreductase; 1.9.3.1). The procedure followed was described by Whorton and Tzagoloff (1967).

Glutamic Oxalacetic Transaminase--(L-aspartate: 2-oxoglutarate aminotransferase; 2.6.1.1). The procedure followed Boehringer Mannheim kit 15923 and Bergmeyer and Bernt (1965).

Acetylcholinesterase--(acetylcholine acetylhydrolase; 3.1.1.7). The procedure followed was described by Ellman, *et al*. (1961).

Alkaline Phosphatase--(orthophosphoric monoester phosphohydrolase; 3.1.3.1). The procedure followed was described by Bessey, *et al*. (1946).

Acid Phosphatase--(orthophosphoric monoester phosphohydrolase; 3.1.3.2). The procedure followed was described by Andersch and Szczypinski (1947).

β-Glucuronidase--(β-D-glucuronide glucuronohydrolase; 3.2.1.31). The procedure followed Sigma kit 325-A and Fishman (1965).

Leucine aminopeptidase--(3.4.1.1). The procedure followed Boehringer Mannheim kit 15793 and Bernt and Bergmeyer (1965).

γ-Glutamyl Transpeptidase--The procedure followed Boehringer Mannheim kit 15794 and Szasz (1969).

NADH-Cytochrome c Reductase--($NADH_2$:cytochrome *c* oxidoreductase; 1.6.2.1). The procedure followed was described by Mackler (1967).

NADH-Cytochrome b_5 *Reductase*--($NADH_2$:cytochrome b_5 oxidoreductase; 1.6.2.2). The procedure followed was described by Strittmatter (1967).

NADPH-Cytochrome c Reductase--($NADPH_2$:cytochrome *c* oxidoreductase; 1.6.2.3). The procedure followed was described by Ernster, *et al*. (1962), and Masters, *et al*. (1967).

NADPH-Dichlorophenolindophenol Reductase--The procedure followed was described by Ernster, *et al*. (1962) and Masters, *et al*. (1967).

Cytochrome b_5--The procedure was essentially the method described by Ernster, *et al*. (1962).

Cytochrome P-450--The procedure followed was described by Omura and Sato (1964).

SECTION V

LABORATORY RESULTS

FATE STUDIES

Volatility

The 5 crude oils in decreasing order of volatility were: Nigerian, Saudi Arabian, Iranian, Empire Mix and Venezuelan. [2]

Saudi Arabian, Nigerian and Iranian crude oils have similar pristane/phytane ratios (0.65 - 0.74) as do Empire Mix and Venezuelan crude oils (1.77 - 1.80). [2]

Fate in Water and Sediments

Saudi Arabian crude oil precipitated or was absorbed out of the water column at a slightly faster rate than was Empire Mix crude oil. Using emulsified Empire Mix crude oil in the presence of sediments, a drastic change in total hydrocarbon distribution in the water column occurred within 96 hours. After 3 hrs exposure, the total aliphatics appeared to have the same hydrocarbon distribution as immediately after emulsion. However, with time, the lower molecular weight straight-chain hydrocarbons were drastically reduced relative to the isoprenoids, pristane and phytane. After 96 hrs, there were a great number of unidentified chromatographic peaks that were not present in the original emulsified oil. In sediments exposed to oil in the water column, alkanes of low molecular weight (n-C11 - n-C20) were present in very low concentrations. The alkanes above n-C20 showed an odd/even dominance that is a typical hydrocarbon distribution for Holocene sediments. The amount of crude oil adsorbed into the sediment from the water column was below the concentration needed to be detected at the natural concentration level of the sediment hydrocarbons (0.0024%).

Both pristane and phytane were present in the same ratios found in Empire Mix crude oil. Transfer of emulsified oil from water column to sediments took place within 96 hrs, but at very low levels. [3-7]

In studies on the transport of oil into the water column from sediments mixed with oil, the oil concentrations in the water remained fairly constant after 3 hrs although the distribution of the hydrocarbons in the water column did change. The lower molecular weight straight-chain hydrocarbons were substantially reduced, relative to pristane and phytane concentrations, and the higher molecular weight hydrocarbons (n-C21 - n-C36) were increased. There was no odd/even hydrocarbon preference which indicates that the distribution of hydrocarbons was not influenced by sediment hydrocarbons. There was an inexplicable 50-fold increase of n-C25 alkane. After 96 hrs, the oil concentration remaining in the sediments completely masked any natural sediment hydrocarbons. Further, there was little change in the distribution and composition of the oil that had been slurried and left in the sediments for 96 hrs. [8-11]

Three classes of compounds, fatty acids, fatty alcohols and hydrocarbons, were chosen to assess the role of sedimentary processes and to investigate their interconversion. Since saturated C15 - C20 isoprenoid hydrocarbons are found in ancient sediments and in petroleums but not in recent sediments, the presence of pristane and phytane, isoprenoids C19 and C20, respectively, were used as indicators of oil pollution in these studies. Sediments from "clean" coastal bays were treated with simulated petroleum pollutants spiked with a ^{14}C labeled C18 acid, alcohol and hydrocarbon. Degradation of the ^{14}C labeled compounds was significant after 60 days. Of the original labeled fatty acids, 50% of the activity was found in the alcohol fraction, 30% in the alkane fraction, and 10% in the fatty acid fraction. Of the original labeled alcohols, 50% of the label was found in the alkane fraction and the remainder in the alcohol fraction. Extracts from the simulated oxygenated sediments showed decreased hydrocarbon/total lipid weight ratio and changed hydrocarbon distribution when compared to the reduced sediments

Fate of Empire Mix Crude Oil With or Without Aeration

The influence of marsh grass (Spartina) on the fate of Empire Mix crude oil incubated with or without aeration was studied for 9 mo. The degradation of straight-chain hydrocarbons was much greater than that of the isoprenoids, and no changes in pristane/phytane ratios were observed under either set of conditions during the first 7 months. After 9 mo, however, the pristane/phytane ratio dropped from an average of 1.80 to 1.57 under aerobic conditions. The n-C17/pristane ratio remained constant in both control and experimental containers without aeration at approximately 1.16. With aeration this ratio dropped drastically with time in the experimental container containing Spartina (0.487 after 9 mo) suggesting increased aerobic microbial activity. Since the ratio with aeration but without Spartina did not change appreciably, it appears that the degradation of n-C17 in relation to pristane by microorganisms was altered by the Spartina. The n-C18/phytane ratio showed similar trends to those described above with the ratio without aeration remaining fairly constant at 1.60, while the ratio with aeration dropped 65%. [12]

Microbial Degradation of Oil

In laboratory studies on the microbial degradation of Empire Mix crude oil, degradation was minimal after 120 hrs of incubation in the absence of added nitrogen and phosphorus. In the presence of added nitrogen and phosphorus, maximum growth, respiration and aliphatic utilization occurred during the first 24 hrs. Visible turbidity and obvious changes in the oil were seen within 48 hrs. The total microbial population experienced an initial decrease during the first 8 hrs, then reached a count of 4×10^8 at 24 hrs and remained essentially constant for the rest of the 120 hr period. Although the stationary phase of growth was reached in 24 hrs, the visual degradation of the oil was not obvious until after 48 hrs. There was a continual decrease in the number and quantity of straight chain aliphatic hydrocarbons with time, and after 66 hrs only the C15, C17, C18, C19 and C20 remained. Only C17, C18 and C19 were present after 120 and 336 hrs and no even or odd chain preference was observed. The branched-chain hydrocarbons decreased but were still present after 120 hrs and pristane and phytane were relatively resistant to attack. The more polar aromatic constituents were metabolized before the less polar compounds. No accumulation of aliphatics occurred in the bacterial cells. [13-21]

Three bacterial cultures were selected for further study on the basis of numerical predominance relative to the total microbial population. Decomposition of filter-sterilized oil by the isolates was studied by analyzing oil after 108 hrs of incubation. Oxygen consumption during the degradation of crude oil was essentially the same for all three isolates, and no alkane specificity was observed. One isolate synthesized pristane and phytane. [22-24]

PHYTOPLANKTON

Toxicity Tests

96-hr Toxicity Tests-

Eighty six 96 ± 3 hr algal toxicity tests consisting of 272 replicates were performed using 5 crude oils on unialgal isolates of 8 representative species of marine phytoplankton. Toxicity was measured in terms of the concentration of crude oil required to reduce cell growth 50% with respect to the controls (EC_{50}). Cell growth was measured in terms of cell numbers. Experimental oil exposure levels ranged from 0 to 100 mg/l assuming 100% emulsification. Actual oil levels present in the growth media were quantitated by IR spectrophotometric analyses of aliquots taken from each bioassay. These values were used to calculate the percentage of emulsion that actually occurred. Nigerian crude oil was the most toxic. Iranian and Empire Mix were intermediate and Saudi Arabian and Venezuelan were the least toxic. In comparing the oils, Empire Mix was approximately twice as toxic as Saudi Arabian to the 5 algae tested with both oils. All 8 species were tested using Empire. *I. galbana* was the most sensitive followed by *S. costatum*, *L. undulatum*, *C. curvisetus*, *Rhizosolenia calcar*, *Asterionella japonica* and *T. decipiens*. *Carteria chuii* was the least sensitive of the 8 algal species tested with Empire Mix. [25-42]

12-Day Toxicity Tests-

Experiments were conducted to determine if extending the exposure time to 12 days would increase the toxic effects of Empire Mix. By extending the exposure time of the organisms to 12 days the Empire Mix increased in toxicity thus lowering the EC_{50} values by more than 5 mg/l in 5 of 7 of the species tested. For *I. galbana*, extended exposure had little effect on the EC_{50}, but *T. decipiens* became more tolerant with extended exposure, and the EC_{50} value increased by 5 mg/l. The greatest effect of extended exposure time was seen at the upper oil levels where cell mortality reached 100% in many cases. [43-50]

Chronic Toxicity Tests-

Thirty-two 96-hr experiments were performed in which *C. curvisetus*, *I. galbana*, *L. undulatum* and *T. decipiens* were tested against Empire Mix and Saudi Arabian. *S. costatum* was tested against all 5 oils. In these studies, phytoplankton exposed to crude oil at concentrations between 0 and 100 mg/l for 96 hrs were subcultured into an oil-free medium. Four of the five species recovered after exposure both to Empire Mix and Saudi Arabian at all concentrations tested. Signs of recovery usually were noted within 14 days with complete recovery within 28 days. *L. undulatum* consistently failed to recover or recovered at a greatly decreased rate in control and treated cultures. [51]

Bioaccumulation

Sixteen experiments were performed using the 7 representative species of marine phytoplankton. Unialgal cultures were grown in inverted, aerated 4-liter serum bottles containing 3 liters of Heinle medium. In each experiment the algae in one bottle were exposed to 4 mg/l Empire Mix (approximately 1/10th of the average amount of oil required to produce 50% reduction in growth in the acute toxicity tests), while the algae in the other bottle were left untreated. Concentrated samples from each bottle were analyzed by LC and GC. Oil-treated algae samples showed consistently higher levels of oil than did the control samples, with some of the oil-treated samples showing oil levels which could account for 40 to 70% of the total amount of oil introduced into the bottles. It is not possible at this time to state whether the oil was being bioaccumulated, adsorbed or absorbed on the algal cells, or whether the algal cells were biologically synthesizing natural oils in response to exposure to Empire Mix. Synthesis appears less probable than either adsorption or absorption. Data suggest that the ability to bioaccumulate, adsorb or absorb oil may be species dependent with *I. galbana* and *T. decipiens* being the most efficient. [52]

ZOOPLANKTON

Toxicity Tests

Data for these tests were based on 98 valid bioassays carried out under static conditions using emulsified oil. Criteria for bioassay validity were: 70% or greater control survival, a 50% Tolerance Limit (TL_m) included within the range of concentrations in the test, and the use of the same dilution scheme as used in all experiments with that test organism. Actual oil levels present in the water were quantitated by IR spectrophotometric analyses of aliquots taken from each bioassay. These values were used to calculate the percentage of emulsion that actually occurred. Toxicity was expressed as TL_m, the level of oil causing 50% mortality of the population in 96 hrs. [53]

A. tonsa, the major primary consumer among the zooplankters, is by far the most abundant copepod in the Mississippi Sound and surrounding Gulf waters, thriving in salinities of 1.1-36.5 o/oo. Of the copepods tested, *A. tonsa* was the most susceptible to crude oil. Of the 5 oils used on cultured *A. tonsa*, Nigerian was the most toxic, Empire Mix, Saudi Arabian and Iranian were intermediate, and Venezuelan was the least toxic. All TL_m values obtained in these tests were low enough that these concentrations of oil can realistically be expected in the water column following a major oil spill. [54-59]

When wild *A. tonsa* (collected from the pilot-plant ecosystem) were exposed to Empire Mix in the laboratory, the resultant TL_m value was the same as that for cultured organisms, indicating that laboratory data on cultured *Acartia* can be extrapolated to *Acartia* in the pilot-plant ecosystem. [60]

Eurytemora affinis is another pelagic copepod found in the estuary of the Mississippi Sound. On the basis of 4 bioassays, this species had an average TL_m of 7.8 mg/l for Empire Mix. [61]

Euterpina acutifrons is the most prominent pelagic harpactacoid copepod found in the Mississippi Sound. As with *A. tonsa*, Nigerian was the most toxic and Venezuelan the least toxic to cultured *Euterpina acutifrons*, with Empire Mix, Saudi Arabian and Iranian intermediate. [62-67]

Cyclops viridis is the predominant species of cyclopoid copepod, usually inhabiting low to moderately saline waters in the estuary. Nigerian was the most toxic of the oils to *C. viridis*. However, the toxicity of the other 4 oils could not be determined reliably, since the concentration of oil had to be so high that the emulsions could not be maintained long enough for testing. [68]

MARSH GRASS

Greenhouse Studies

The majority of the studies on the uptake of oil by marsh vegetations was conducted using potted plants in the greenhouse. When clumps of *Juncus roemerianus* and *Spartina cynosuroides* (the dominant species in the Mississippi marshes) were exposed to Empire Mix, they showed an increase in hydrocarbon content, both aliphatic and aromatic. The small amount in the control plants presumably was due either to the natural waxy substances synthesized by plants or to prior exposure to oil. There was a greater concentration of aromatic compounds than there was aliphatic compounds in control plants. On the basis of aliphatic hydrocarbons, it appeared that *Spartina* did not readily take up oil. These and other later data from similar experiments using Saudi Arabian also indicated that, in general, *Juncus* took up oil more readily than *Spartina*. The higher level of residues detected in dead plants and in roots of live plants suggested that oil was being absorbed rather than bioaccumulated. [69-72]

Field Studies

A series of plot experiments were conducted on a natural marsh located on a marsh island west of St. Louis Bay, Mississippi. The effects of single and multiple exposures of marsh plants to doses of 250, 600, 750 and 1500 ml of Empire Mix for various periods, ranging from 4 mo to 2 yrs, was studied using 1 m^2 plots subdivided into 4 subplots (0.25 m^2). [73-74]

From the results of these experiments, a number of conclusions were drawn concerning the effects of the exposure of marsh plants to Empire Mix:

(1) Single exposures to doses of 250 ml or above caused some plant mortality as well as reduced growth the following year ranging from 40% (250 ml/m^2) to 90% (1500 ml/m^2). [75-78]

(2) Multiple spills of doses of 250 ml or greater, 12 mo apart, significantly reduced plant growth the following growing season from 23% (250 ml/m^2) to 94% (1500 ml/m^2). The degree of reduction increased as the dosage increased. [79-83]

(3) Following single exposures to doses of 250 ml and greater, marsh plants contained detectable oil residues up to 9 mo after exposure. Following oil exposure, the roots of live marsh plants contained more oil residues than the shoots; the bottom of the shoots contained more oil residues than the tops of the shoots; and dead marsh plants contained more oil residues than live plants. [84-88]

(5) The decomposition of dead marsh plants subjected to the ebb and flow of natural tides was reduced as much as 40 to 50% after 1 yr of exposure. The oil-contaminated plant material will remain in the environment longer, thus increasing the opportunities for oil to enter the estuarine food chain via the detritus pathway. [89]

MULLET

Acute Toxicity

A number of toxicity tests using 130-160 mm mullet were conducted to: (1) compare the relative toxicities of Empire Mix, Saudi Arabian, Venezuelan, Nigerian and Iranian crude oils to mullet; (2) determine the levels of Empire Mix which would be used for short-term laboratory tests to study the effects of oil on the enzyme system of mullet; and (3) determine the dosage of Empire Mix to be spilled on the pilot-plant ecosystem during the chronic field studies.

Of the oils tested against mullet, Nigerian (100% mortality at 200 mg/l) and Saudi Arabian (100% mortality at 350 mg/l) were the most toxic, Iranian and Venezuelan were intermediate (TL_m's between 400-800 mg/l) and Empire Mix (TL_m > 800 mg/l) was the least toxic. [90]

Based on these results, laboratory treatment levels of 75 mg/l or less were chosen for short-term enzymological studies. In addition, it was estimated that the levels being considered for use in the field studies (5-20 mg/l) would allow long-term survival of the mullet.

Behavior

There was a distinct difference in the behavior of mullet when exposed to different crude oils. During exposures of up to 800 mg/l to the less toxic oils (*i.e.*, Empire Mix, Venezuelan and Iranian), the movements of the mullet appeared generally normal to somewhat suppressed (they were often in a stationary position near the top of the tank). When exposed to Nigerian and Saudi Arabian in 114-liter aquaria at concentrations ranging from 200 to 400 mg/l, the mullet almost immediately became hyperactive. During early stages of exposure they swam rapidly in a straight line, often hitting the sides of the test tanks. Rapid swimming movements often were followed by a pause during which the mullet rapidly flexed their bodies laterally in a shaking motion. Near death, there was a loss of equilibrium during which mullet sank to the bottom of the test tank.

Enzymology

Mullet enzymes typically were assayed in mitochondrial or supernatant fractions, or whole homogenates of brain, gill, liver and muscle tissues. More than 1700 individual enzyme assays were conducted, using over 1000 mullet.

The following enzymes were studied in juvenile mullet exposed to 75 mg/l emulsified Empire Mix for 96 hrs: lactic dehydrogenase (LDH), malic dehydrogenase (MDH), NADPH-cytochrome c reductase (CYT RD), cytochrome oxidase (CYT OX), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), acetylcholinesterase (CHE), alkaline phosphatase (ALP), acid phosphatase (ACP), β-glucuronidase (GLU) and leucine aminopeptidase (LAP). With the exception of GLU, the enzymes did not appear to be affected by this oil exposure. Although muscle and brain were the tissues expected to be least affected by oil exposure, they were the 2 tissues in which there was a change in enzyme level, with a statistically significant decrease in mitochondrial GLU activity from muscle and brain. There were no differences observed following oil exposure for any enzymes studied in either liver or gill tissue. It had been expected that gill and liver would be the tissues most likely to be affected since these tissues are involved with initial contact (gill) and detoxification (liver). [91-94]

Several enzymes were studied in juvenile mullet 96 hrs after intraperitoneal injection with 100 μl of Empire Mix. There were indications of changes in the activities of CYT OX, CYT RD, GOT, GPT and LAP in some tissues. No changes were noted in GLU, LDH, MDH or α-hydroxybutyric acid dehydrogenase. [95]

To determine if any direct interaction between molecular components of oil and the enzyme molecule existed, which could lead to activation or inhibition, studies of in vitro time-dependent and time-independent effects were conducted. Empire Mix in concentrations up to 80 mg/l was incubated with homogenates of mullet liver, and the enzyme GOT was monitored. This enzyme was selected since it was part of the assay system for MDH, which had shown positive responses to oil in other studies. No in vitro inhibition or activation of GOT in the presence of oil was observed. [96-97]

During the wide spectrum enzyme studies discussed above, one microsomal oxidase, NADPH-cytochrome c reductase, gave indications of induction in livers of mullet following a one-week exposure to Empire Mix. To test the inductive potential of crude oil on microsomal hydroxylation enzymes, mullet were exposed to 75 mg/l of Empire Mix or Saudi Arabian for 96 hrs. Liver weight to body weight ratios and hepatic protein were also measured. Liver weight increased by about 50% in response to both oils. Concentrations of both total hepatic protein and microsomal protein were depressed, particularly by Saudi Arabian; this apparent decline represents the failure of protein to increase at the same rate as liver size. Both cytochromes b_5 and P-450 were induced above control levels, particularly cytochrome P-450. NADPH-dichlorophenolindophenol reductase was increased following exposure to both oils and NADPH-cytochrome c reductase following exposure to Empire Mix; these increases may have been activations rather than inductions. NADH-cytochrome c and NADH-cytochrome b_5 reductase activities were not affected, but remained proportional to the protein level. Although Saudi Arabian had a greater effect on liver size and hepatic protein content, Empire Mix had a greater effect on enzyme levels. Therefore, both crude oils exert physiological effects with potential pharmacological significance in mullet. [98]

In conclusion, the most consistent effects following oil exposure in mullet were in those enzymes associated with stress and/or detoxication. β-Glucuronidase was depressed and some microsomal oxidases were activated or induced following exposure to crude oil.

Fatty Acids

Mullet were exposed to 10, 20, 30 and 75 mg/l emulsified Empire Mix for 96 hrs. Fatty acids 14:0, 16:0, 16:1 and 22:6 showed some changes in the oil-treated mullet, but no consistent dosage-response pattern of either increases or decreases was evident. The mean percent fatty acid composition of the shorter-chain fatty acids up to 18:0 in fish exposed to 75 mg/l oil were higher than the control fish. However, the longer-chain fatty acids were lower in the treated fish than in the control fish. In addition, there were differences between laboratory controls and wild mullet for 11 of the 25 fatty acids reported. [99-101]

Seasonal variation was observed with higher percentages of long-chain polyunsaturated fatty acids present during the colder months, and predominance of short-chain fatty acids present during the warmer months. [102-103]

Oil Uptake

The LC and GC results of numerous oil uptake tests exposing mullet to various concentrations of emulsified Empire Mix crude oil for varying periods of time were extremely erratic. Even so, certain trends were evident. Fish which were dead when removed from the test tanks usually were higher in oil content than live fish exposed under similar conditions. Insofar as partitioning of the oil into various tissues is concerned, gill and gut tissues almost always contained oil. Approximately 60% of the liver tissues examined contained measurable amounts of oil. It should be noted that a number of the brain samples contained oil. Muscle tissues were generally low or negative in oil content. As would be expected the gill tissues taken early in the experiments were higher in oil content than those taken later. [104]

For all of the above tests, oil in the tissues was calculated using both the LC and GC analyses. The amount of oil found in each sample by the two methods differed since the calculation of oil was based on the aromatic hydrocarbons for the LC analyses and on the aliphatic hydrocarbon fraction for the GC analyses. However, the trends of oil uptake and depuration for each tissue were similar using either method, with the exception that for a given sample low levels of oil could often be detected using the LC, while none could be detected from a portion of the same sample using the GC.

Histology

No histological changes could be attributed directly to the effect of either acute or short-term chronic exposures of mullet to Empire Mix and Saudi Arabian. However, bacterial infections (accompanied by the pathological conditions associated with them) were observed in the majority of tests that lasted more than two wks.

Laboratory Studies on Disease

One unexpected result observed when oil was spilled on the pilot-plant ecosystem was the sudden and dramatic outbreak of fin rot which occurred in the oil-treated ponds. This was especially surprising since fin rot had not been observed during numerous acute laboratory tests nor had reference to such an occurrence in response to oil been encountered in the literature.

The lack of disease in the laboratory experiments was thought to be due to the short (96 hr) duration of the majority of these tests. Consequently, chronic laboratory experiments were initiated in an attempt to determine if the occurrence of fin rot in the first pilot-plant study was indeed related to the exposure of mullet to oils or simply a unique happening due to some peculiar order of events or circumstances. In these control laboratory studies both Empire Mix crude oil and Saudi Arabian crude oil in concentrations ranging from 14 mg/l to 75 mg/l were shown to cause the disease. Overall, the incidence of infection was 7.1%, 97.1% and 100% for control, Empire Mix-exposed and Saudi Arabian-exposed mullet, respectively. [105]

Bacteriological examinations from both field and laboratory mullet (approximately 425 fish sampled) indicate that primarily 5-6 different colonial types of bacteria constitute the normal microflora on the exterior of the mullet in the control situations while only 1-2 colonial types are predominant in the microflora on the exterior of the mullet exposed to oil. It is important to note that the suspected pathogen identified as *Vibrio* is also found on the exterior of some control fish, as well as the oil-tested fish but in much fewer numbers. [106]

Bacteriological examination of the kidneys of the mullet indicated that under normal conditions the kidney was sterile. Only from kidneys of fish which were heavily infected with fin rot were any bacteria isolated. The kidney isolate was always in pure culture and had identical characteristics of the predominant isolate (Vibrio) from the exterior of the infected mullet.

Results from the microbial examination of the intestines of the mullet were less clear cut. There appeared to be no normal bacterial flora in the intestines and the number of organisms present seemed to vary from fish to fish.

Pure culture isolates from both healthy and diseased mullet were examined for their ability to utilize crude and degraded oil and for their hemolytic ability. Interestingly, the organism identified as Vibrio was the only hemolytic organism isolated which was capable of utilizing either crude or degraded oil. Although this did not fully establish the relationship between microbial utilization of oil and the ability to cause disease, it did suggest a connection between the two observations. [107]

The data seem to indicate that the potentially pathogenic bacteria are a normal part of the mullets' microflora and only cause a problem when one or more of the mullets' defense mechanisms are rendered inoperative. To further test this theory, the mucus was removed by mechanical means from the tails of a number of mullet. These test organisms contracted fin rot in 6-10 days. In addition, the tail of a number of mullet were scraped near their base causing superficial lesions. Again these mullet contracted fin rot.

In summary, these limited laboratory tests clearly establish the relationship between oil exposure and the occurrence of fin rot in mullet. However, the mechanism has not as yet been elucidated.

SHRIMP

Acute Toxicity

The purposes for running acute toxicity tests with shrimp were the same as those stated for the acute toxicity tests using mullet.

The ranking of the 5 oils in decreasing toxicity to shrimp was: Nigerian, Empire Mix, Saudi Arabian, Iranian and Venezuelan. Nigerian was the most toxic as 10 mg/1 resulted in 100% mortality of the test shrimp. The TL_m values were estimated to be between 15 and 25 mg/1 for Empire Mix, Saudi Arabian and Iranian, and between 35 and 45 mg/1 for Venezuelan. [108]

When the toxicity tests with mullet, oysters and shrimp were evaluated, it was obvious that shrimp were the most sensitive to all 5 oils. Therefore, a calculated concentration of about 4 mg/1 was chosen as the concentration of Empire Mix to be spilled in the pilot-plant ecosystem, since the majority of the shrimp population should survive this dosage. In addition, 2, 4 and 8 mg/1 were chosen as the dosages of choice for laboratory studies using shrimp.

Behavior

Generally, the first and the most characteristic behavioral response of shrimp exposed to crude oils was a spiraling motion from the bottom to the top of the tank followed by a loss of equilibrium. After falling back to the bottom of the tank they often remained on their sides, rapidly moving their swimmerettes which sometimes resulted in "scooting" movements but usually caused no forward motion. The swimming motions of the treated shrimp often were rapid and erratic. These reactions were essentially the same for all oils studied; however, their onset came early and were more pronounced with Nigerian than with the other crude oils when tested at the same dosage. This was to be expected since, to shrimp, Nigerian was the most toxic of the 5 oils.

Enzymology

Enzymes were assayed in mitochondrial and supernatant fractions of hepatopancreas homogenates. In excess of 1100 individual enzyme assays using more than 750 shrimp were conducted. The following enzymes were assayed from shrimp exposed to 8 mg/1 emulsified Empire Mix for 12 hrs: acid phosphatase (ACP), malic dehydrogenase (MDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), β-glucuronidase (GLU), cytochrome oxidase (CYT OX) and NADPH-cytochrome _c_ reductase (CYT RD). There were no detectable effects from oil exposure on the enzymes assayed. [109]

Shrimp were exposed to 4 mg/l emulsified Empire Mix for 24 and 48 hrs. The following results were indicated: mitochondrial and supernatant γ-glutamyl transpeptidase (GGTP) and leucine aminopeptidase (LAP), and supernatant CYT OX were decreased after oil exposure; mitochondrial and supernatant GOT and GPT, supernatant CYT RD and mitochondrial CYT OX were elevated after oil exposure; ACP, ALP, and GLU, were not consistently affected. [110]

Shrimp were exposed to 10 mg/l emulsified Empire Mix or Saudi Arabian for 24 hrs. The activities of the following enzymes were determined: ACP, ALP, CYT OX, CYT RD, GLU, GOT, GPT, GGTP, LAP, MDH, α-hydroxybutyric dehydrogenase (HBDH) and lactic dehydrogenase (LDH). Enzyme activity responses were different following the *in vivo* exposure to the two crude oils. Exposure to Empire Mix resulted in no significant differences in the enzyme activities between oil-treated and control groups. However, some dramatic results were observed with Saudi Arabian. In the mitochondrial fraction, ALP, GGTP and CYT OX activities were decreased. In the supernatant fraction, ALP, GGTP and LAP activities were decreased. The following activities were increased in the supernatant fraction: HBDH, GOT, GPT, LDH, GLU, and MDH. Mitochondrial HBDH activity was increased. The other enzymes were unaffected by oil exposure. Although supernatant increases in activity were observed for several enzymes, in most cases concomitant decreases in mitochondrial specific activities were not observed. [111-112]

No statistically significant induction of the microsomal oxidase, NADPH-cytochrome *c* reductase, was observed in shrimp hepatopancreas following up to 24 hrs of exposure to either Empire Mix or Saudi Arabian. [113]

In conclusion, the enzyme activities of the hepatopancreas do not seem to be greatly affected following *in vivo* exposure of brown shrimp to Empire Mix, whereas many of the same enzyme activities are greatly altered following exposure of the shrimp to Saudi Arabian.

The following enzymes were assayed in mitochondrial and supernatant fractions of whole body homogenates of grass shrimp (*Palaemonetes spp*.) exposed to 50 mg/l emulsified Empire Mix for up to 12 days: MDH, LDH, HBDH, GPT, GGTP, LAP and creatine phosphokinase (CPK). There were no statistical differences observed with the 7 enzymes tested. When ratios of supernatant/mitochondrial specific activities for each sample were compared statistically, only the creatine phosphokinase ratios showed a statistical difference. [114-115]

Fatty Acids

Fatty acid concentrations were determined in muscle tissue of shrimp exposed to 15 mg/l emulsified Empire Mix for 96 hrs. Fatty acids 16:1 and 18:3 were higher and 20:5 was lower in oil-exposed shrimp muscle than in control shrimp muscle. [116-118]

Shrimp were exposed to 2, 4 or 8 mg/l Empire Mix for 12-24 and 48-96 hrs, and fatty acids of muscle and hepatopancreas were determined. In muscle tissue of shrimp exposed to 8 mg/l Empire Mix for 12-24 hrs, the fatty acids 14:1, 15:1 and 17:1 were higher than in control shrimp muscle. Fatty acids 14:0, 14:1, 15:0, 16:0, 17:0 and 18:1 showed a progressive increase in mean percentage with increased oil dosage. Fatty acid 17:1 was higher in muscle of shrimp from all three oil doses than in control tissue. There were fewer differences in fatty acids between control and oil-exposed shrimp at 48-96 hrs than at 12-24 hrs. At 48-96 hrs of exposure, fatty acid 15:1 of muscle from shrimp exposed to 2 mg/l was higher than the control. In hepatopancreas samples, fatty acids 18:1, 18:2 and 20:0 were significantly higher and 20:1 was significantly lower in shrimp exposed to these doses of oil for 12-24 hrs. No significant differences were observed after 48-96 hrs of exposure. [119-122]

In shrimp exposed to oil, there was a pattern of higher mean percentages for short-chain fatty acids and lower mean percentages for long-chain unsaturated fatty acids following exposure. Possible explanations are: (1) oil disrupted the normal chain elongation saturation-desaturation process; (2) organisms were stressed, and selectively β-oxidized the longer chain fats for energy; and (3) oil could cause an enzymatic catabolism of the bonds in the longer-chain carbon acids, resulting in higher mean percentages of short-chain acids and less long-chain fatty acids. Differences in the fatty acid pattern decreased with increased exposure time, indicating some form of compensation or acclimation to the stress. This decrease correlated positively with the decline in hydrocarbon content of the tissues with time.

Oil Uptake

Shrimp were exposed to different levels of emulsified Empire Mix for 96 hrs with samples taken every 12 hrs for the first 24 hrs and at 24 hr intervals thereafter. By LC, shrimp muscle contained higher levels of oil at higher dosage levels (2-8 mg/l) during the first 24 hrs, followed by lower tissue levels of oil uptake for the next 72 hrs at all dosage levels. The level of oil as determined by LC in the shrimp hepatopancreas increased for the first 24-48 hrs, after which time a decrease in oil content was observed. Using GC analyses the data were very erratic and did not demonstrate the presence of oil in the hepatopancreas. [123-124]

Histology and Disease

Acute Studies-

Histological findings as a result of short-term laboratory exposure of shrimp to Empire Mix were inconclusive. However, a number of interesting observations were made.

The exposure of shrimp to 2 mg/l Empire Mix in 114-liter test aquaria for 72-96 hrs resulted in the development of black spot gill syndrome. Grossly, the gills of the treated shrimp were dark brown to black in color. The gills of moderately involved specimens had a mottled appearance; while in more severely affected shrimp, the entire gill appeared dark. Histologically, there were numerous branchiae which appeared to be swollen and/or fused. In these areas the epithelial lining of the lamellae were thickened, and the cytoplasm appeared to be swollen with a brownish infiltrate. Some of the dark areas, especially near the base of the branchiae, were very dense with little structural detail. It was thought that this syndrome was caused directly by the oil treatment. However, additional tests at the same treatment levels failed to consistently cause the syndrome. In addition, the black gill syndrome also developed in shrimp which were being held prior to testing; in this instance it appeared that a bacterium (tentatively identified as Vibrio spp.) appeared to be responsible. In conclusion, it is not possible at this time to state whether the development of the black gill syndrome in the treated shrimp was a direct response to oil or the indirect effect of the oil acting as a stressor stimulating a bacterial infection which caused the syndrome. However, it seems probable that the syndrome is a general response of the gill tissue which can be triggered by a number of factors. [125]

Laboratory Feeding Experiments-

A series of experiments were conducted using aerated, 114-liter aquaria with sand covered bottoms in which test shrimp were fed 5 g of oil-treated feed (250 mg/l) per day while the control shrimp were fed 5 g of feed with no oil added. At the termination of the experiment, some of the shrimp were sacrificed and examined histologically. A number of abnormal areas were observed in the area of the mandibles, gastric mill and along the inner edge of the carapace, especially ventrally near the muscles supporting the digestive track attached to the body wall. The lesions typically involved the keratinized layers and were characterized by a break or erosion of the carapace sometimes accompanied by a poliferation of cells from beneath the carapace.

In all cases, the necrotic areas were stained brownish and very closely resembled the necrotic areas observed in the black gill syndrome. The necrotic areas were often located either between the two layers of the lining of the digestive tract or just beneath it. It is not known by what means these lesions arise. Since only one lesion was observed in the controls, it would seem likely that they were caused, at least indirectly, by oil. Since the food was granular it could have caused mechanical abrasion thus allowing oil to get into sensitive areas. [126]

OYSTERS

Acute Toxicity

Acute 96-hr toxicity tests indicated that oysters were very tolerant to crude oil. There was no significant mortality caused by Empire Mix at exposure levels of up to 800 mg/l, nor was there significant mortality caused by Saudi Arabian, Nigerian, Iranian or Venezuelan at exposure levels of up to 400 mg/l. There was some random low-level mortality of controls and test organisms not associated with oil exposure. [127]

Behavior

The only noticeable behavior observed in oysters when tested with crude oil was their tendency to close when initially exposed. Later, as the oil began to degrade, and after the concentration of water solubles decreased, some oysters would open and begin to pump. According to the results obtained in oil uptake studies, some oysters remained closed or did not pump significantly during the duration of the test. This lack of consistency complicated the interpretation of the data obtained in uptake and depuration studies. It was thought that this behavior could be countered by pegging the oysters open. However, the uptake data were still erratic, indicating that some did not pump even when forced to remain open.

Depuration

Although Empire Mix was not acutely toxic to oysters in concentrations which could be expected in the environment; the oil, if taken in, could be expected to affect the taste of the oyster and thus potentially to impact the economically important commercial oyster industry. Oysters were exposed to 300 mg/l Empire Mix for 96 hr and then placed in baskets in a clean estuary. Routine taste tests of these oysters indicated that they retained an oily taste for at least 9 wks.

Enzymology

More than 2400 individual enzyme assays were conducted on more than 300 oysters. Enzymes were assayed in fractions of whole body homogenates of oysters exposed to 75 mg/l emulsified Empire Mix for up to 7 days. No statistically significant differences were observed between control and oil-treated oysters in the specific activities of acetylcholinesterase (CHE), acid phosphatase (ACP), alkaline phosphatase (ALP), cytochrome oxidase (CYT OX), glutamic oxaloacetic transaminase (GOT), mitochondrial leucine aminopeptidase (LAP) and supernatant malic dehydrogenase (MDH) A number of enzyme activities from oysters exposed to Empire Mix were statistically different from those of controls. However, no clear trend as to stimulation or inhibition was observed. At 7 days, the ß-glucuronidase (GLU) activity was significantly lower than the activity at 4 days. With glutamic pyruvic transaminase (GPT), the supernatant activity peaked at day 2, and the mitochondrial enzyme activity was significantly higher at day 7. The soluble LAP activity was lowest on day 2 of treatment. Mitochondrial MDH activities dropped on day 2, but by day 7 were in the range of the control activity. This pattern was the reverse of what was observed with the other enzymes. The enzyme patterns observed may be the result of the normal response of an organism to an environmental challenge. Another possible explanation may be related to the very rapid decrease in the available concentration of oil in the water column. [128-129]

Oysters were exposed to a surface film equivalent to 100 mg/1 of Empire Mix daily for 7 days and were then sampled at 2, 3, 4, 5, 7 and 8 days after cessation of oil additions. There appeared to be an elevation of both mitochondrial and supernatant GOT and GPT at 2 days after cessation of oil addition. Mitochondrial LAP appeared elevated at 2, 5, 7 and 8 days. Supernatant LAP and creatine phosphokinase (CPK) appear unaffected by oil exposure. Although certain elevations and depressions in enzyme activity were observed, the only clear pattern of response was the general increase noted at 2 days post-treatment for all enzyme activities except supernatant LAP. [130]

Enzyme activities were measured in oysters exposed to 75 mg/1 Saudi Arabian, Nigerian, Iranian and Venezuelan for 4 days. The following enzymes were studied: ACP, ALP, CHE, CYT OX, GLU, GPT, GOT, LAP and MDH. Overall, there were minimal effects on the enzymes tested. No statistically significant differences were observed between the controls and organisms exposed to Saudi Arabian. Nigerian caused decreases in supernatant activities of GOT, GPT and LAP. Iranian caused an increase in mitochondrial GPT and supernatant LAP. Venezuelan caused no statistically significant changes. [131-132]

NADPH-cytochrome c reductase was studied in fractions of whole body homogenates of oysters exposed to 75 mg/l emulsified Empire Mix, Saudi Arabian, Venezuelan, Nigerian and Iranian. There were statistically significant increases in NADPH-cytochrome c reductase activity after exposures to Empire Mix and Nigerian. [133]

In conclusion, oysters that had been treated with Empire Mix, either emulsified or a surface film, showed few significant effects on the activities of the enzymes tested. Of the enzymes that were affected by acute oil exposure, most are related to carbohydrate metabolism. MDH is involved in both aerobic and anaerobic metabolism in the oyster, and it may be hypothesized that under conditions of acute oil exposure the oyster closes and functions anaerobically. The trend of decreased activity at 2 and 4 days of exposure with the return to normal at 7 days probably represents a normal compensation by the oyster to oil stress. Carbohydrate (tricarboxylic acid cycle) and amino acid metabolism are enzymatically connected by GOT and GPT. GLU is involved also in carbohydrate metabolism. Increased levels of LAP in the oyster could indicate that under anaerobic conditions there is mobilization of tissue proteins for energy.

Enzyme activities of oysters exposed to Nigerian and Iranian demonstrated some changes, whereas those from oysters exposed to Saudi Arabian and Venezuelan were unaffected. Induction of a microsomal oxidase, of potential significance in detoxication, was caused by Empire Mix and Nigerian.

Fatty Acids

The fatty acid composition of oysters exposed to 75 mg/l emulsified Empire Mix for 24 and 72 hrs were determined. There were statistical differences between groups for acids 17:1 and 22:1, but no outstanding trends between control and oil-exposed oysters were apparent. [134-135]

Oil Uptake

The oysters were exposed to 75 ppm Empire Mix oil for 72 hrs, then transferred to a clean tank and allowed to depurate for 96 hrs. Data on oil uptake in oysters were extremely erratic apparently due to variations in pumping habits. Nevertheless, the trend was toward a constant increase in oil concentration with increased length of exposure to the oil. In a second system, the oysters were exposed to different amounts of both Empire Mix and Saudi Arabian oils. The concentration of oil increased with an increase in concentration with the uptake of Empire Mix being higher than Saudi Arabian. The concentration of oil in the oysters was calculated almost totally by LC since the GC was unable to detect oil in such low amounts. [136-137]

Histology and Disease

Laboratory exposure of oysters to Empire Mix produced no histological changes that could be directly associated with their treatment with Empire Mix.

SECTION V(a)
FIELD RESULTS

TIDAL-POND STUDY

Description of the System

On July 25, 1973, 57 liters of Empire Mix crude oil were spilled on a 9 x 9 m shallow tidal pond situated at the Gulf Coast Research Laboratory, Ocean Springs, MS. The spill dosage was calculated to yield 250 mg/l oil at low tide. A similar adjoining pond served as control. The pre-spill conditions of the ponds were: salinity 10-12 o/oo, temperature 30-37 C, and dissolved oxygen 3.7 mg/l at low tide and 12.2 mg/l at high tide. The dikes were vegetated by *Juncus roemerianus*, *Spartina cynosuroides*, *Spartina alterniflora* and *Distichlis spicata*.

Within 2 hrs after the spill, the pond was completely covered by oil. After 5 days, the oil had dissipated and was concentrated along the bank, on the mud and on the marsh plants. Infrared spectrophotometry of the water 1 day after the spill indicated an oil concentration of 32.5 mg/l.

Observations on the fate of oil in the sediments and effects on floral and faunal populations were made for 18 mo.

Fate of Oil in Sediments

During the first 18 days following the oil spill, the oil migrated downward from the sediment surface to a depth of 42 cm. No changes occurred in the less volatile, higher molecular weight aliphatic hydrocarbons which were detectable by the technique employed. [138]

After 4 mo the percentage of *n*-C10 - *n*-C20 hydrocarbons as compared to the *n*-C21 - *n*-C33 hydrocarbons had dropped approximately 8% in the top 2 cm of sediments. The ratios of n-C17/pristane and n-C18/phytane had decreased by 12%. In the 25-34 cm core section, the ratio of percentage of low molecular weight to high molecular weight hydrocarbons was reduced about 35%. The n-C17/pristane and n-C18/phytane ratios in the deeper core sections had increased as compared to the top sections. [139-140]

Only small changes occurred in the composition of the crude oil between 4 mo and 7 mo. Using the known ratios of n-C17/pristane and n-C18/phytane in the crude oil and the hydrocarbon distribution between n-C11 and n-C23 as indicators of the presence of oil, the top section of the core (1-13 cm) still demonstrated considerable evidence of oil. The n-C17/pristane, n-C18/phytane, and pristane/phytane ratios were close to those in the Empire Mix crude oil itself. The sediments contained slightly less oil at 7 mo than at 4 mo. The n-C17/pristane and n-C18/phytane ratios increased in these core sections, indicating that the natural level of n-C17 and n-C18 hydrocarbons present were more dominant than those in oil. The deepest core section analyzed (30-42 cm) contained only trace amounts of oil. [141]

Samples collected 12 mo after the oil spill indicated that the percent hydrocarbon/lipid weight decreased with depth in both ponds as expected in recent sediments. The oil-spill pond sediments showed a slightly higher concentration of hydrocarbons than the control pond sediments, although the level of crude-oil hydrocarbons in the oil-spill pond sediments after 1 yr was extremely low. The relative percent of hydrocarbons below n-C22 in both control and oil-spill pond was less than 10 percent. The oil-spill pond hydrocarbons had a significantly higher pristane/phytane ratio than the control pond hydrocarbons. The pristane/phytane ratio of the oil-spill pond was similar to that in Empire Mix crude oil. This ratio, though different in each pond, remained fairly constant to a depth of 42 cm. [142]

The final core samples, taken from the experimental pond 18 mo after the oil spill, showed little evidence of crude oil in either the top 13 cm or the bottom 13 cm. Both sections contained 19 mg/l aliphatic hydrocarbons (based on dry sediment weight). The top section of sediment contained a higher percentage of total aromatic hydrocarbons than the bottom section.

Phytoplankton/Zooplankton

Observations 30 days after the spill indicated a lower phytoplankton population and a higher zooplankton population in the oil ponds than in the control ponds. The immediate killing of the oil-sensitive zooplankton by the initial oil spill allowed phytoplankton to replicate without predation. As the oil dissipated from the ponds, the zooplankton recovered in the presence of an abundant standing crop of phytoplankton. The zooplankton population then rapidly increased, reducing the phytoplankton population appropriately. This left an abundant zooplankton population composed of animals in their developing stages. These observations were supported in part by the large numbers of copepod naupliars found in the samples and were consistent with the laboratory data. Six and seven mo after the oil spill, the zooplankton populations in both ponds were similar in respect to numbers and diversity of species. Phytoplankton, however, were more abundant and slightly more diverse in the control pond. By 9-10 mo after the oil spill, zooplankton and phytoplankton were in greater abundance and diversity in the oil pond than in the control pond. By 12 mo after the oil spill, phytoplankton were more abundant in the oil pond (15 species) than in the control pond (11 species). An opposite trend was seen with zooplankton, with greater abundance found in the control pond. No difference in diversity was seen.

Primary productivity measured by *in situ* light and dark bottle technique revealed a 43-65% reduction in the oil planktonic community 16 days after the spill; respiration values were also reduced by 16-18%. The decrease in primary productivity may be due to a decrease in phytoplankton biomass up to 30 days after the spill rather than to any physiological inhibition of photosynthesis. About 2 mo after the oil spill, the difference in primary productivity of the plankton community between oil and control ponds was less than 20%, which was about the condition of the two ponds prior to the oil spill. [143-144]

Marsh Grass

The results obtained during this tidal pond study paralleled the results reported for the field studies discussed earlier (SECTION V). Plants from both the control pond and the treated pond contained aliphatic and aromatic hydrocarbons. The oil caused plant mortality and reduced the plant growth in subsequent mo. The hydrocarbon content of the dead plant material was greater than that in the live plants, and the roots of live plants contained more hydrocarbon than did the shoots. [145-146]

Animals

The amount of meaningful data obtainable on the changes in the populations of organisms present in the tidal ponds at the time of the oil spill was extremely limited because the ponds were stocked by natural recruitment prior to their enclosure and because they were subject to flooding during periods of high water. Seine samples prior to the oil spill indicated that the most predominant species present in the ponds were menhaden (*Brevoortia spp.*) and grass shrimp (*Palaemonetes spp.*). Present in relatively low numbers were the bay anchovy (*Anchoa mitchilli*), the silverside (*Menidia beryllina*), the striped mullet (*Mugil cephalus*), the pinfish (*Lagodon rhomboides*) and the ladyfish (*Elops saurus*). Seine samples following the spill indicated an initial drop in the menhaden and grass shrimp populations followed by a repopulation after about 2 mo. The most dramatic effect on the fish population appeared to be the predation of menhaden (which were swimming erratically in response to the oil) by the ladyfish. [147]

Significantly higher concentrations of oil were found in oil-treated organisms than in control organisms. The molluscs showed the highest uptake (mussels and snails), crabs the next highest, and fish and shrimp the least. The difference in feeding habits of these animals may be responsible for the variation in oil uptake. [148]

FIRST PILOT-PLANT ECOSYSTEM STUDY

Description of the System

The pilot-plant ecosystem (Figures 1 and 2) was built at the NASA National Space Technology Laboratories (NSTL) in Hancock County, Mississippi.

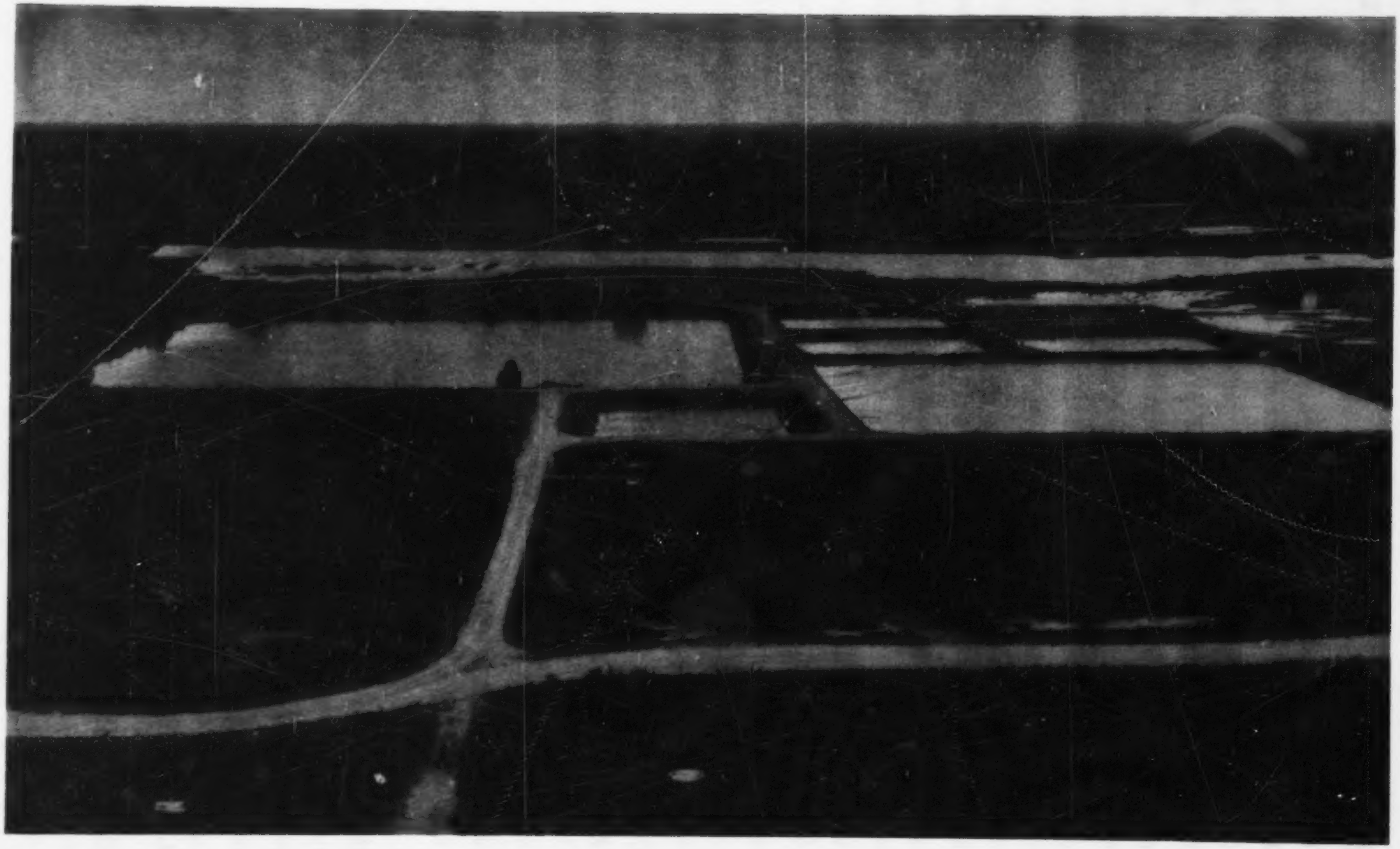

Figure 1. Aerial photograph of the pilot-plant ecosystem located at the National Science Technology Laboratory in Hancock County, Mississippi.

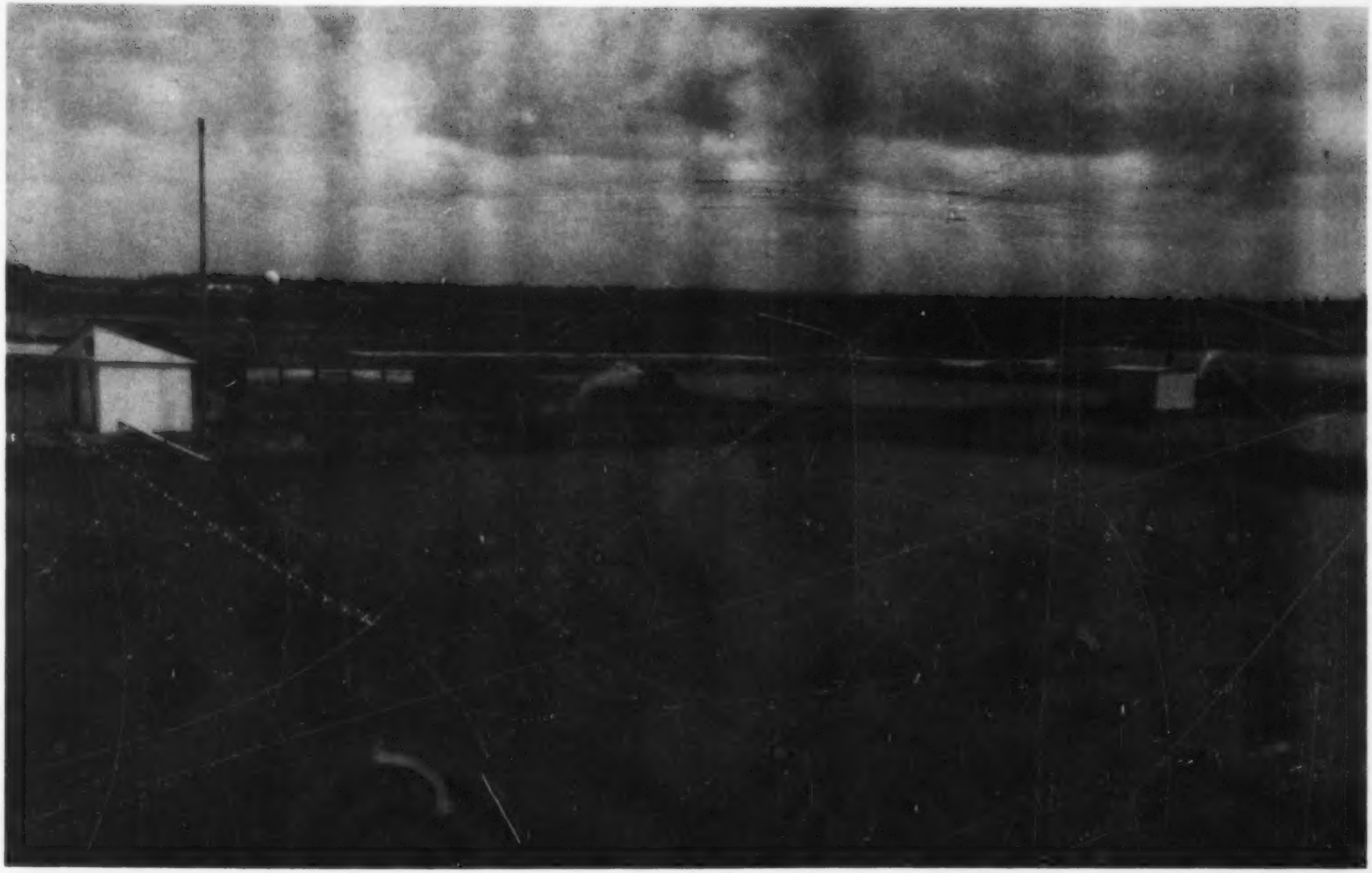

Figure 2. Ground level photograph taken midway in the development of the pilot-plant ecosystem ponds used in this study. At the time this picture was taken, only one pumping system was functional and the marsh grass had not been planted around the edges of the ponds.

It consisted of 4 ponds measuring 46 x 46 m at the top and narrowing to 30 x 30 m at the bottom. An overflow pipe was located approximately 2.5 m from the bottom. In June 1972, the ponds were filled using seawater (approximately salinity 28 o/oo) from the Gulf of Mexico mixed with fresh well water to adjust the final salinity to about 14 o/oo.

Clumps of Juncus mixed occasionally with other species (e.g., Spartina, Scirpus and Distichlis) were dug from preselected areas in St. Louis Bay marsh, transported to NSTL and immediately transplanted along one side of each pond. The clumps included the natural muddy substrate and all the benthic fauna and microflora associated with it. Figure 3 is a photograph of the marsh grass 6 mo after transplantation. Two additional sides of each pond were planted with marsh plants in November 1974. The marsh grass grew extremely well, and at the termination of the project it was approximately 1.2-1.5 m tall and had completely covered the pond banks. Additionally, a rich organic sediment had developed in the ponds prior to their use.

A tidal simulation system (TSS) was designed and installed between ponds 2 and 5 which were to be used as control ponds and 3 and 4 which were to be used as treatment ponds (Figure 4). The TSS was operated in such a fashion that 46 cm of water was pumped from one pond to the other every 12 hrs and then the flow was reversed.

The overflow pipes in the two ponds to which oil was to be added were fitted with a wooden box containing polyurethane to absorb any oil contained in run-off waters.

The salinity in all ponds eventually dropped to about 6 o/oo as the result of rainfall and the continual mixing caused by the operation of the TSS.

Barges originally planned for obtaining seawater were no longer available; consequently, it was decided that the salinity would be increased by the addition of commercial salts. Calculations were made to determine the

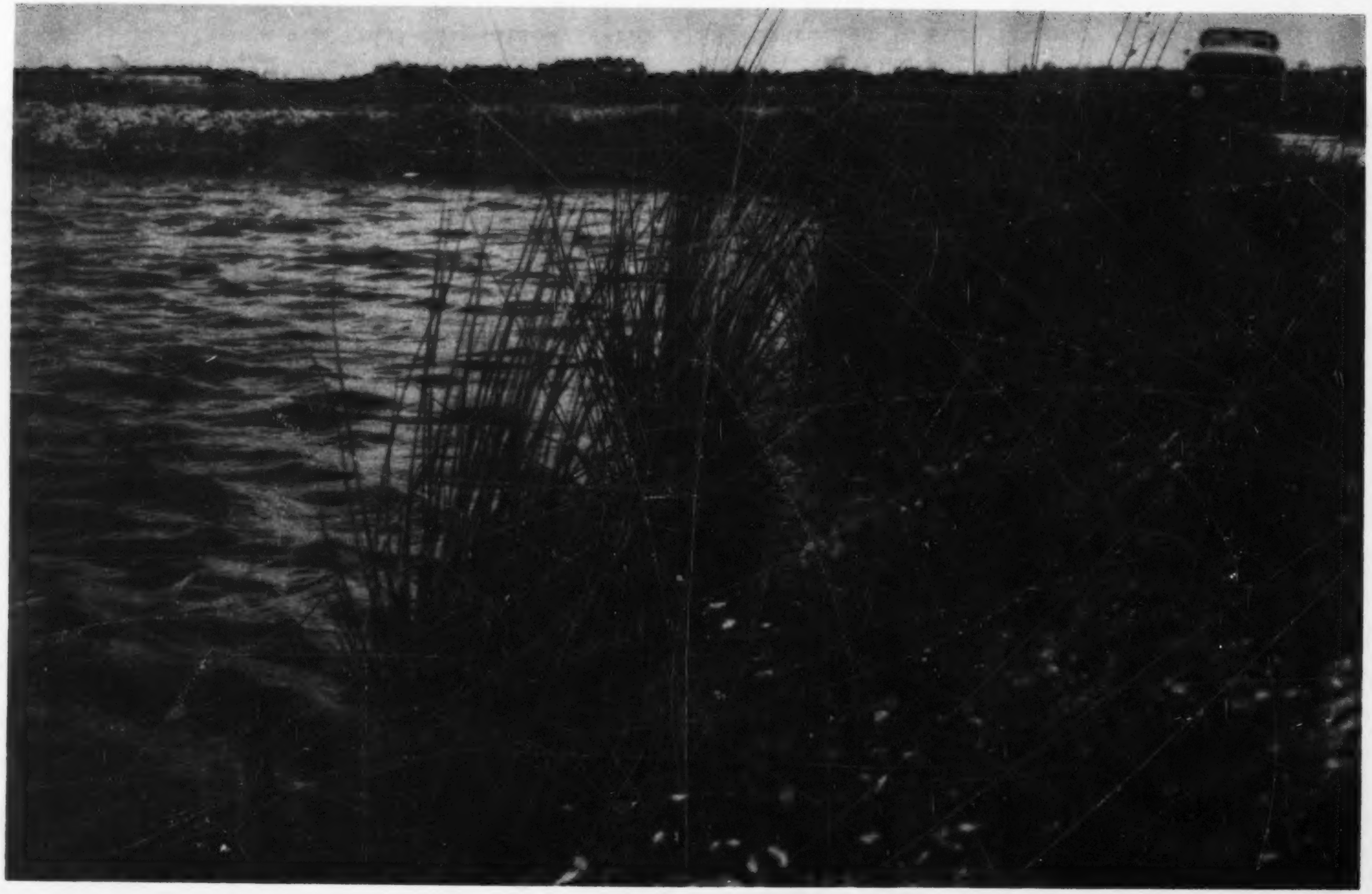

Figure 3. Photograph showing clumps of *Juncus* growing along the edge of one experimental pond in the pilot-plant ecosystem.

Figure 4. Tidal-Simulation System (TSS) installed in Pump House.

amounts of Na, K, Ca, Mg, Cl and SO_4 required to increase the salinity to 12 o/oo based on the ratios of these ions in Rila Sea Salts Mix which was used routinely in the laboratory. The salts added were NaCl, $CaCl_2$, $MgSO_4$, $MgCl_2$ and KCl.

In November 1973, approximately 360 mullet, 750 brown shrimp and 360 oysters were added to each pond. The oysters were placed on the bottom along one side of each pond.

Conduct of the Oil Spill

On 17 July 1974, 11.3 liters of Empire Mix were added to each test pond (ponds 3 and 4). The additions of oil were made in the following manner. Three 3.8 liter plastic jugs without tops and tied to a large brick were filled with oil, perforated in the bottom and sunk in the middle of pond 3 at 10:45 a.m. A small amount of the oil was spilled onto the surface during the additions. For a period of hrs following the spill, oil seeped up to the surface from the sunken jugs and was distributed around the pond by wind action. At the outset all of the oil was blown almost directly to the northwest corner of the pond. Within 3 hrs, the wind had shifted to a southeasterly direction and the oil slick moved across the pond. By late afternoon approximately 3/4 of the pond surface was covered with a thin layer of oil, and oil was visible around edges of the pond. Similarly, oil was added to pond 4 at 3:00 p.m. on the same day. At that time, and for several hrs thereafter, no wind was evident; and the oil spread rather evenly in all directions from the center of the pond (Figure 5).

On 19 July, oil was visible around the edges of the pond but very little remained on the surface; the small quantities of oil visible on the surface were located predominantly on the south side of the pond. Only a small amount of residual oil was left in the jugs when they were removed from the ponds. Using the procedure described above, an additional 11.3 liters of oil was added to pond 3 at 10:00 a.m. and 11.3 liters of oil was added to pond 4 at 10:30 a.m. The ponds were continually monitored for dissolved oxygen content and no observable differences were noted between the treated and control ponds.

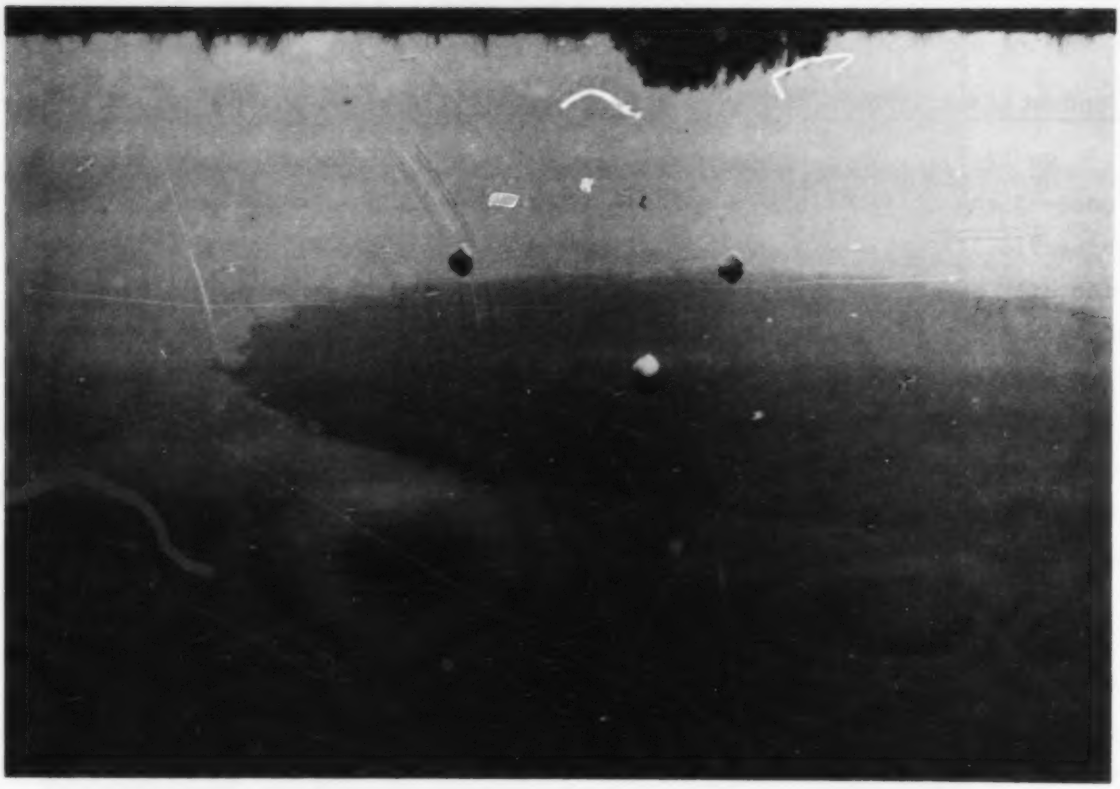

Figure 5. Photograph taken shortly after oil spillage in one of the pilot-plant ecosystem ponds. Note ring of oil near the three floats in the center of the pond.

Infrared analysis of water samples taken immediately prior to the second oil spill indicated a concentration of between 0.16 and 0.32 mg/l total oil in the water column. In terms of the amount of oil added to the ponds, the following calculations should be of interest. If the total amount of oil added to the ponds had been evenly distributed onto the surface, an oil slick of 0.01 - 0.02 mm in thickness would have resulted. On the other hand, if all of the oil added to the ponds had been equally distributed throughout the water column, the concentration of oil in the water column would have been approximately 4.0 mg/l. Based upon the infrared analyses, if a uniform concentration of oil throughout the ponds could be assumed, the concentration of oil in the ponds would have been 0.2 mg/l.

Small patches of oil were visible on the surfaces of the ponds for 7 days after the final oil additions were made. Furthermore, oil was noticeable on the edges of the ponds and on the marsh grass. It is highly significant to note that the waters in the two oil ponds became extremely clear as compared to the control ponds. It was possible to see for considerable distances under water in these ponds while visibility was limited to 0.3-0.6 m in the control ponds. With time, very little evidence of the oil spill was observable although small oil-stained areas remained on the banks for several weeks after the oil spill. The presence of oil in the sediments of the ponds was confirmed when the cast net was dragged on the bottom of the ponds; small bubbles of oil rose to the surface and then quickly dissipated.

Overall, it was concluded that the oil spill operation, as conducted, was highly successful in terms of getting reasonably uniform distribution of oil in the ponds.

Key Events

The chronological order of key events during this study were:

12 December 1973	First Pre-spill sampling
7 February 1974	Second Pre-spill sampling
1 June 1974	Approximately 475,000 l of water was removed from each of the four ponds and replaced with seawater (salinity, 14 o/oo).

11 June 1974	Third Pre-spill sampling
24 June 1974	Salts added to ponds
17 July 1974	11.3 l of Empire Mix added to each test pond (3 & 4)
19 July 1974	another 11.3 l of Empire Mix added to each test pond (3 & 4)
28 July 1974	First Post-spill sampling
19 August 1974	Second Post-spill sampling
24 September 1974	Third Post-spill sampling
6 November 1974	Fourth Post-spill sampling
9 January 1975	Fifth Post-spill sampling
11 March 1975	Sixth Post-spill sampling
6 May 1975	Seventh Post-spill sampling
16 May - 2 June 1975	Experiment terminated

Environmental Data

Daily recordings of barometric pressure, temperature, relative humidity and rainfall were obtained from NASA for the immediate area. No unusual events occurred during the test period.

The salinity was maintained between 6 and 10 o/oo for the duration of the study by the additions of seawater and/or commercial salts. The dissolved oxygen ranged between 6 and 10 mg/l.

Fate Studies

The first pre-spill core samples of the sediment showed a fairly large concentration of straight-chain hydrocarbons below *n*-C23 in all four ponds, indicating a contribution from source material other than terrestrial plants. One likely source would be algae, which contribute large amounts of *n*-C17 to the organic matter in the sediments. Pristane and phytane were present in all sediments, but the pristane/phytane ratios were not typical of most crude oils. Pond 2 (control) exhibited a completely different profile from the other three ponds. All ponds contained trace quantities of hydrocarbons as shown by infrared spectrophotometry; Ponds 2 (control), 4 (oil) and 5 (control) had 0.025 mg/l hydrocarbons while Pond 3 (oil) had 0.038 mg/l hydrocarbons. [149-153]

Six mo later (just prior to the oil spill) surface grab samples of sediment taken from the ponds in the vicinities of the first core samples were black and contained hydrogen sulfide indicating that they were in extremely reducing environments.

Hydrocarbon analyses of sediments from Pond 2 (control) showed large concentrations of n-C15 and n-C17 and a greater concentration of higher molecular weight alkanes (C25, C27, C29, C31). These observations were probably a reflection of the occurrence of an algal bloom and the presence of considerable marsh plant debris.

In Pond 5 (control) the distribution of higher molecular weight hydrocarbons remained the same; however, there was an increase in the concentration of lower molecular weight hydrocarbons. This pond (5) contained the highest total hydrocarbon concentrations of any of the 4 ponds.

The vast quantities of grasses, observed growing in Pond 3 (oil) were reflected in the hydrocarbon analyses. Also, the low molecular weight hydrocarbons usually contributed by the blue-green algae had decreased.

The samples from Pond 4 (oil) had a rather thick algal mat (3-4 cm) and n-C17 was the dominant hydrocarbon. There appeared to be a loss of higher plant contribution to this pond.

Even though some changes in total hydrocarbon concentrations and distributions occurred in the ponds, it is interesting to note that the percent lipid/dry weight of sediment was about the same in all four ponds. [154-158]

Eleven days after the initial oil spill there was a slight oil sheen at the edge of both treated ponds. Core samples oozed oil. The mud samples taken from the east bank of the ponds were black and contained hydrogen sulfide. Hydrocarbon content was exceedingly high in the sediments from Pond 4 (oil). Gas chromatographic analysis indicated petroleum hydrocarbons in Pond 4 (oil) whereas the chromatograms for sediments in Pond 3 (oil) indicated the presence of high concentrations of algal mat material.

Sediment samples taken from the middle of the ponds one mo after the oil spill indicated that a small percentage of the crude oil had reached these sediments since the percent hydrocarbon/lipid weight was slightly higher in the test ponds than it was in the control ponds. It became apparent that the oil was not evenly distributed throughout the sediments and consequently it was impossible to accurately follow the fate of the oil in the sediments. Apparently, the actions of the pumps greatly influenced the results. [159-164]

Consequently, the final sediment samples were composites of 4 separate samples taken 9 mo after the oil spill. Sediments from the half of the pond nearest the pump were combined, and those opposite the pump were combined. In Pond 3 (oil), the total hydrocarbons in the sediments nearest the pump represented 42 mg/l, whereas the total hydrocarbons in sediments opposite the pump yielded 1080 mg/l. The organic matter near the pump was sparse and was in an oyster shell hash, while samples from the opposite side were a dark clay mud. Neither the mud nor the shell hash had a high amount of extractable lipid material (both less than 1%), although the sediments opposite the pump contained 5 times as much as the sediments near the pump. The chromatograms contained a very large envelope with only a small percentage of normal hydrocarbons resolved. The sample nearest the pump showed a distribution of aliphatic hydrocarbons more characteristic of young sediments. In this sample the odd-numbered carbons (n-C21, n-C23, n-C25, n-C27, n-C29, n-C31) predominated. In the sample opposite the pump, however, the envelope contained only a few resolved peaks with no odd-carbon dominance. In Pond 4 (oil), the sediments nearest the pump contained 654 mg/l total hydrocarbons, whereas the sediments opposite the pump contained 100 mg/l. Again, the percent of extractable lipid material represented less than one percent in these samples. GC results indicated that the sediments opposite the pump were the least changed from the last sampling period. These sediments yielded the most typical odd/even-carbon number ratio of hydrocarbons in the higher molecular weight range, i.e. strong odd-carbon number preference. These sediments also appeared to contain a significant amount of algae (n-C17 present in high concentration). A large unresolved envelope was present in the n-C17 - n-C25 molecular weight range. The unresolved envelope was not in the same molecular weight range as that of Empire Mix, and there was little evidence that Empire Mix was present in these sediments. GC results indicated large amounts of unresolved hydrocarbon material. [165]

Microorganisms

The total bacterial population in the water column of the ponds and the sediments fluctuated from sample to sample, but there was no discernable difference due to the oil spill. Furthermore, no significant shifts in the composition of the microbial population were observed as determined by tests conducted for yeasts, fungi, actinomycetes, hydrocarbon-utilizing/sulfate-reducing bacteria and hydrocarbon-utilizing/nitrate-reducing bacteria. The ratio of hydrocarbon-utilizers to total microbial population was essentially the same for all 4 ponds and was not affected by the oil spill. [166-167]

Microbiological observations made in association with disease in mullet are reported in a later section on Mullet Disease.

Phytoplankton/Zooplankton

No quantitative differences were observed in the phytoplankton populations existing in the control and oil-treated ponds as a result of the spilling of Empire Mix on 17-19 of July 1974. Increases in numbers of phytoplankton were observed in all 4 ponds after the oil spills. Since these increases occurred in the control ponds as well as the oil ponds, they must be attributed to some environmental factor other than the oil. [168-172]

Prior to the oil spill there was a mixed zooplankton population in all ponds with *Acartia* (copepod) and *Brachionus* (rotifer) predominating. Immediately following the oil spill there was a sharp decline in the total zooplankton population in the oil-treated ponds followed by a gradual return to pre-spill levels during the next 20 days. Significantly, after this time *Brachionus* was clearly the dominant species. Since these changes did not occur in the control ponds, the decline in the *Brachionus* and

Acartia populations with a subsequent shift to a Brachionus dominated population must be attributed to the presence of the Empire Mix crude oil. [168-172]

Core Sampling-

Core samples were taken from the ponds on a regular basis beginning in September of 1974. Prior to that time, a few core samples had been taken at irregular intervals. Over 115 total core samples were taken.

The samples taken from Ponds 2 (control) and 4 (oil) after the first oil spill were grown in liquid and agar media. Bold's Basal Media (BBM) was used to support freshwater algae, and Heinle's Media at a salinity of 20 o/oo was used for culture of saltwater algae. Mud was taken from the top and bottom of each core. Liquid cultures were counted as to cells/ml. Agar plates were counted as to number of colonies on the plate. Presumably, each colony represented a single cell falling on the agar when mud was sprayed on the plate.

The analysis of the core sample data indicated little or no pattern of algal population growth. Though cores were always taken in the same corner of each pond, no relationship existed between the numbers of algae in cores taken two weeks apart. It had been assumed that algae were spread evenly in the mud of the ponds, and that a decline in population in one area represented a decline in the entire benthic population. This was disproved by taking 2 cores 1 m apart on several sampling dates and comparing the results. No relationship was observed between the numbers of algae grown from duplicate cores.

In conclusion, the areas of concentration and paucity of algal populations present in the mud are rather like a checkerboard in their distribution. It is not known if this situation is unique to the experimental ponds.

Marsh Grass

Due to the small quantities of Empire Mix spilled and due to its uneven distribution along the pond banks, no meaningful conclusions could be drawn from this phase of the study. There was very little plant mortality associated with the spill; and, while the plants in the oil-treated ponds contained more oil than the plants from the control pond, there was a considerable difference in the amounts depending upon the location in the treated ponds from which the plants were taken. By 125 days there was no significant difference in the oil residue levels contained in the plants taken from the treated or control ponds.

Oil Uptake

The analyses of several shrimp and mullet samples collected just prior to the addition of the second 11.3 liters of oil to the test ponds indicated oil uptake. All samples collected after that time, including the first post-spill sampling period, failed to indicate oil in any of the samples of shrimp, mullet or oysters by the analytical techniques employed (GC analyses and LC analyses using a fixed wavelength UV detector at 254 nm). Subsequent samples were archived and analyzed later using a Schoeffel GM-770 variable wavelength UV detector at 277 nm. While the number of samples in most cases was small, certain tentative conclusions can be drawn.

Mullet-

Brain samples collected throughout the 9 1/2-mo study all contained increased concentrations of aromatic compounds while very few gill samples had measurable increases in aromatics. Increased concentrations of aromatics were evident in liver samples collected 11 days after the spill but were not noticeable in the liver samples collected for the following 4 mo probably due to the small number of samples analyzed. Liver samples collected for the remainder of the study showed considerable increases in aromatics. [173]

It should be pointed out that the aromatic hydrocarbon profile of the mullet tissue samples changed during the course of the study. There was a general shift to shorter retention times for the predominant peaks in the samples from the oil-treated ponds thus indicating an increase in polarity of those compounds. This could be a reflection of the degradation of the aromatics into phenolic, diol, or epoxy-like compounds.

Shrimp-

The data on the oil content of shrimp hepatopancreas and muscle were inconsistent, due in part to the small number of samples available for analysis. Of 21 post-spill muscle samples (pooled samples, 5 organisms per sample) oil was indicated in 4 samples. Of 22 post-spill hepatopancreas samples (pooled samples, 5 per sample) 8 samples showed an increased amount of aromatic compounds. The significance of these data is the fact that oil was found in the hepatopancreas of shrimp, 5 1/2 mo after the oil spill. This would indicate that the shrimp are obtaining the oil from the environment (sediments). [174]

Oysters-

The concentration of oil in the oysters, even at the first sampling period was low. This was not surprising since the concentration of oil in the water column was extremely low. However, after 3 1/2 mo only 25-33% of the oysters from the test ponds had any traces of oil in them, thus confirming the generally accepted fact that the oysters purge themselves of oil with time. [175]

Enzymes

For the majority of the 10 sampling periods, the following enzymes were assayed: acetylcholinesterase (CHE), alkaline phosphatase (ALP), β-glucuronidase (GLU), glutamic pyruvic transaminase (GPT), malic dehydrogenase (MDH) and lactic dehydrogenase (LDH). Enzymes were assayed in mitochondrial and post-mitochondrial supernatant fractions of whole oysters, shrimp hepatopancreas, and mullet brain, gill, liver and muscle homogenates. These enzymes were selected on the basis of laboratory data indicating effects related to oil treatment. It was hoped that CHE might give indications of nervous and muscular function, ALP changes in membrane transport, GLU conjugate breakdown and hormone usage, GPT energy source shifts and protein metabolism, MDH aerobic metabolism and LDH anaerobic metabolism.

Much variability was apparent with many of the samples. Also some seasonal variation in specific activities occurred with most of the enzymes studied.

Mullet-

In mullet, elevations of muscle CHE immediately after the oil spill could indicate increased nervous and muscular activity in response to the presence of the noxious chemicals. Occasional decreases in brain CHE activity may be a reflection of the oil-treated animals' poor health. [176-177]

There were generally no effects of oil exposure on supernatant ALP activities from any of the organisms studied. Mitochondrial activity in brain, liver and gill appeared depressed on several occasions, while muscle mitochondrial activity showed both elevations and depressions during the course of the experiment. The results do not correlate with the changes seen in the individual mullet experiment, which will be discussed below. The significance of these changes in activity is not clear. Similarly, the laboratory experiments did not result in changes in ALP activities in mullet following oil exposure. [178-181]

There was no effect on GLU by oil exposure in brain or the supernatant fraction of gill. Gill mitochondrial activity was depressed very late in the experiment. Liver activity in both fractions appeared depressed immediately after the oil spill and may have been elevated very late in the experiment. Muscle supernatant GLU may have been elevated on several occasions following the oil spill. Elevations in activity could indicate stress in that the enzyme could activate circulating hormone conjugates at the tissue level. GLU is the only enzyme in mullet shown to be affected by acute oil exposure in the laboratory in which muscle mitochondrial levels were decreased and brain mitochondrial levels increased. Differences in laboratory and field results might very well reflect the difference between acute and chronic effects and the time required for hormonally-mediated reactions to stress to become manifest. [182-185]

There were no oil-mediated effects on MDH in gill mitochondria and brain, gill and liver supernatant. On a few occasions, mitochondrial MDH from brain and muscle appeared elevated, supernatant MDH from muscle appeared depressed and mitochondrial MDH from liver showed both an increase and decrease. The changes observed may indicate some effect upon the animals' rate of aerobic metabolism. [186-189]

LDH was not affected by oil in any of the tissues observed. This correlates with the laboratory results. [190-193]

GPT levels were not affected in brain or liver mitochondria or gill or muscle supernatant. GPT levels in brain supernatant and gill and muscle mitochondria were elevated during the early samplings after the oil spill. Liver supernatant GPT appeared rather consistently depressed following the oil spill. Those latter results could indicate alterations in nitrogen metabolism in the oil-treated organisms. [194-197]

Typically, the results of the pond oil exposures regarding changes in enzyme activities in mullet were not dramatic. This correlated with the results of laboratory studies. The results here on pooled tissue samples are not as clear-cut as the results obtained with individual mullet, where the individual health and condition could be better correlated with enzyme activities. Some of the particularly aberrant data points can be correlated with mullet samples that show very pronounced disease symptoms. In general, the results indicate that some of the enzyme systems of the mullet are responding to their stressful situation.

Twenty days after the first oil spill, enzymes were assayed in tissues of individual mullet in an attempt to provide closer correlations among biochemical, physiological and pathological parameters than was possible with the usual pooled samples.

Liver weight to body weight ratios were increased in the organisms from the oil-treated ponds over those from the control ponds. Variability was low between the two ponds within each group. Similar increases were observed in laboratory studies and are consistent with observations of liver hypertrophy induced by certain xenobiotics in other organisms. The lack of differences between the two ponds in each group with regard to liver weight to body weight ratios indicates that the increases observed in the test ponds were caused by an oil-related phenomenon and not general nutritional state. However, total length to body weight ratios were not consistent between the two groups. The ratios from the oil-treated ponds were higher than the ratio from Pond 2 (control) but lower than that from Pond 5 (control). The ratios, which should give an insight into the nutritional condition and/or general health of the fish, indicate that the fish from Pond 5 may have been suffering from a nutritional deficiency, and that the fish from Pond 2 were the healthiest. [198]

ALP was increased over control values in gill and muscle. The enzyme appeared decreased in liver mitochondria and unaltered in liver supernatant. The fish from one of the control ponds (5), which may have been under a nutritional stress, had higher ALP in the gill and muscle than the fish from the other control pond (2). Likewise, the fish from Pond 4 (oil), which had a more severe disease condition, had higher ALP in the gill and muscle than the fish from the other oil pond (3). These elevated ALP levels in the oil-treated fish could indicate an effect of oil on membrane permeability. In addition the enzyme could be an index of the degree of stress the organism is experiencing, since changes in this enzyme have been noted in stress-related work in mammalian systems. [198]

GLU was not affected by oil in either the gill or the liver. In both fractions of muscle the activities of the organisms from the oil-treated ponds were higher than those from the control ponds. Muscle mitochondrial GLU from one control pond (5) fish was higher than that taken from the other control pond (2). GLU, a hormonally-controlled enzyme, could be an indication of stress in muscle tissue. GLU hydrolyzes glucuronic acid conjugates, which may allow the circulating forms of some hormones to enter the effector tissues. Thus, elevated GLU activities could indicate a greater utilization of stress-mediated hormones in peripheral tissues. [198]

CHE in the muscle was not affected by oil exposure. [198]

MDH was not affected in the gills or in the liver mitochondria. MDH was depressed in the liver supernatant and elevated in both fractions of muscle in the oil-treated fish. These results indicate changes in the metabolic activities of tissue and their level of aerobic metabolism in response to stress, although the changes in MDH do not seem directly proportional to the degree of stress the organisms were experiencing. [198]

Generally, the results of the individual mullet tissue enzyme assays showed some differences not apparent in the pooled mullet tissue enzyme assays. There may have been a masking effect by pooling tissues. Although some trends were indicated, no definite correlations between biochemical and pathological parameters could be made. Also, differences in mullet samples from the pond were more pronounced than were observed with mullet in laboratory studies. It is possible that two of the enzymes studied here, ALP and GLU, are potential indices of stress phenomena.

Shrimp-

In shrimp hepatopancreas, there were no oil effects on ALP, MDH, or GPT activities. GLU and LDH were depressed on some sampling occasions in the organisms exposed to oil. No significant effects of treatment with Empire Mix on enzymes in shrimp were observed in the laboratory which is in agreement with the few enzyme effects observed in these field tests. However, these few changes observed may be an indication of a long-term effect on some physiological process in shrimp by exposure to Empire Mix. [199-203]

Oysters-

Generally, the oysters from the oil ponds did not have significantly different enzyme activities than those from the control ponds. Differences between control and oil-treated oysters at a few of the sampling periods were observed with CHE, ALP, MDH and GPT. There was no effect on GLU. This is unlike the laboratory studies in which the majority of crude oil-mediated effects on enzyme activities were demonstrable in oysters. However, the changes observed indicate that certain biochemical and physiological processes within oysters may be affected for several mo after exposure to Empire Mix crude oil. [204-208]

Fatty Acids

Fatty acid methyl ester weight data from all tissue samples were highly variable and precluded their use for analysis. For this reason, all analyses are based on fatty acid percent composition of samples using a Duncan's New Multiple Range Test (DNMRT).

Mullet-

Of the mullet tissues examined, only liver showed any appreciable change in fatty acid profile. Fatty acid 16:0 decreased as in shrimp muscle tissue but with no difference found between control and oil-exposed fish. Unlike shrimp muscle tissue, there was no increase in the percent concentration of 16:1 fatty acid. The mullet muscle also differed from the shrimp muscle tissue in that there was an increase in 18:0 with no real difference in control and treatment tissues. These reactions of mullet tissue are unexplainable, unless the trend was chain elongation from the 16C fatty acid to the 18C fraction. When the sum of all saturated fatty acids was examined and compared to control liver tissue, there was a variable but general decrease for both control and oil-treatment tissue. [209-210]

Shrimp-

Of the shrimp tissue examined in the pond studies, only shrimp muscle revealed any real differences between control- and oil-exposed organisms. In chain length there was a trend toward chain elongation upon oil exposure. Klenk and Kremer (1960) saw a chain elongation procedure in fish in certain conditions, and Knipprath and Mean (1966) showed elongation under temperature stress. The true significance of this finding is not clear but could be a stress response.

Perhaps more pronounced than the above phenomenon was the trend toward unsaturation of fatty acids upon chronic exposure of the shrimp to oil conditions. The tissue from oil-exposed organisms showed a more rapid decline in the concentration of the 16:0 fatty acid than tissue from control organisms. There was a similar reduction in the saturated 18-carbon fatty acid. When the sum of the 18:2 and 18:3 acids was plotted, there seemed to be a direct relationship with the reduction in 18:0. This observation suggested an increased conversion of 18:0 to 18:2 and 18:3 upon chronic exposure to oil. The pattern toward greater unsaturation may be a stress response. [211-213]

There was also evidence of an increase in 20:2, 20:4 and 20:5 fatty acids with increased time after exposure, but the increase did not appear to be related to oil exposure or with any decrease in 20:0 or 20:1 acids.

When chain length and degree of saturation were examined in the hepatopancreas tissue of shrimp, no statistical differences of biological variation could be found between control and oil-exposed tissue.

Oysters-

In the examination of fatty acids from oysters, the same trend toward a decrease in 16:0 and 18:0 acids was found. One interesting but inconsistent phenomenon, when all organisms were compared, was the initial increase in these acids after oil was spilled. After the third post-spill sampling, the percent concentration of these two FA's was on a straight-line decrease. No single unsaturated fatty acid could be singled out that could account for the decrease in percent content of the saturated acids, but when total saturation was tabulated, the decrease was highly significant. [214-216]

Conclusions-

There was a definite statistical decrease in 16:0 acid in oysters, shrimp muscle and mullet liver tissue. The importance of this phenomenon is not known, but others (Klenk and Kremer, 1960; Knipprath and Mead, 1966; Vale, et al., 1970) have seen a similar pattern in response to stress. The oyster and shrimp muscle tissue also showed a decrease in 18:0 FA. Oddly, the mullet liver showed an increase in the 18:0 fatty acid. Vale, et al. (1970) saw a similar response in tainted fish fillets. In general, there was a movement toward unsaturation of fatty acids during the period of the pond studies. This trend was seen in both the control- and oil-treated organisms. The oil-exposed shrimp muscle showed this tendency to a statistically (P<05) greater extent than control tissue.

Pathology

Mullet-

During the early part of the first field study, no histological changes were observed which could be associated with the exposure of the mullet to oil except the normal conditions associated with a bacterial infection (described below). Subtle changes may have appeared in the gill and liver tissues about 4-6 mo post-spill. However, when the ponds were harvested, after 11 mo, 20 mullet from each pond were preserved for histological study. Two significant conditions were observed. There was a high incidence of enlarged, swollen, clubbed and fused gills in the treated fish. In addition, nearly all of the livers from treated mullet had enlarged hepatocytes accompanied by a reduction in sinusoidal spaces. There also appeared to be a loss of granulation with increased vacuolation as compared to the control mullet. It seems significant that these two conditions were not observed until late in the study, especially since the uptake studies indicated that "oil" began to appear in the mullet liver samples late into the study period. [217]

Shrimp-

During this estuarine-pond study in which Empire Mix was spilled, no disease conditions directly attributable to oil were found in the shrimp. The shrimp were in the ponds for 2 winters. During the early part of the second winter, numerous shrimp with black spots on their bodies were observed in all ponds. No shrimp were caught during the early spring sampling or when the ponds were harvested in May.

Oysters-

No pathological conditions associated with exposure to Empire Mix were observed in oysters during these field studies. The lack of response during this study cannot be considered conclusive because of complications with the experimental design. The oysters had been placed on the bottom on the sloping sides of the ponds. They did extremely well for 6-8 mo (pre-spill) as they were fat and had new shell growth. However, with time they settled into the mud which apparently interfered with their feeding. Mid-way into the treatment period it was noted that the general health of the oysters in all ponds was declining. Their tissues were clear and watery, and mortality was high. All pond studies on oysters were terminated in January (7 mo post-spill) due to the above conditions.

Disease in Mullet

An unexpected occurrence following the oil spill was the outbreak of a severe case of fin rot in the population of mullet in the oil-treated ponds (Figure 6). By the first sampling (13 days post-spill), all fish from the oil-treated ponds had moderate to severely eroded fins, loose scales, numerous petechiae and small lesions as well as active sites of infection; most were very emaciated. The microbial counts in the water of all four ponds were low (100-1000/ml), but 40-55% of the organisms obtained from the oil ponds (3 and 4) were hemolytic while only 23-24% of the organisms obtained from the control ponds were hemolytic. By the 28th day post-spill and during subsequent samplings the fish from the treated ponds no longer had active sites of infection, and most had fins in various stages of regeneration. Hemorrhaging was less evident; however, their scales dislodged much easier when handled than did the controls. When the experiment was terminated (11 mo), the majority of the fish harvested were found to have regeneration marks on their fins indicating that the original infection included most of the oil-exposed mullet. It was a general observation by all personnel involved that the oil-treated fish (even late in the study when they outwardly appeared to be recovering) were much more susceptible to handling stresses. Fish taken from all ponds at this latter stage of the study would initially appear normal. However, after an hr or so in aerated holding vats the oil-exposed fish would develop numerous hemorrhages and would often die while the fish from the control pond remained normal in appearance. [218-220]

SECOND PILOT-PLANT ECOSYSTEM STUDY

Description of the System

It was decided that more information might be obtained in a second pilot-plant ecosystem study by testing 3 different crude oils rather than repeating the original study using only Empire Mix crude oil.

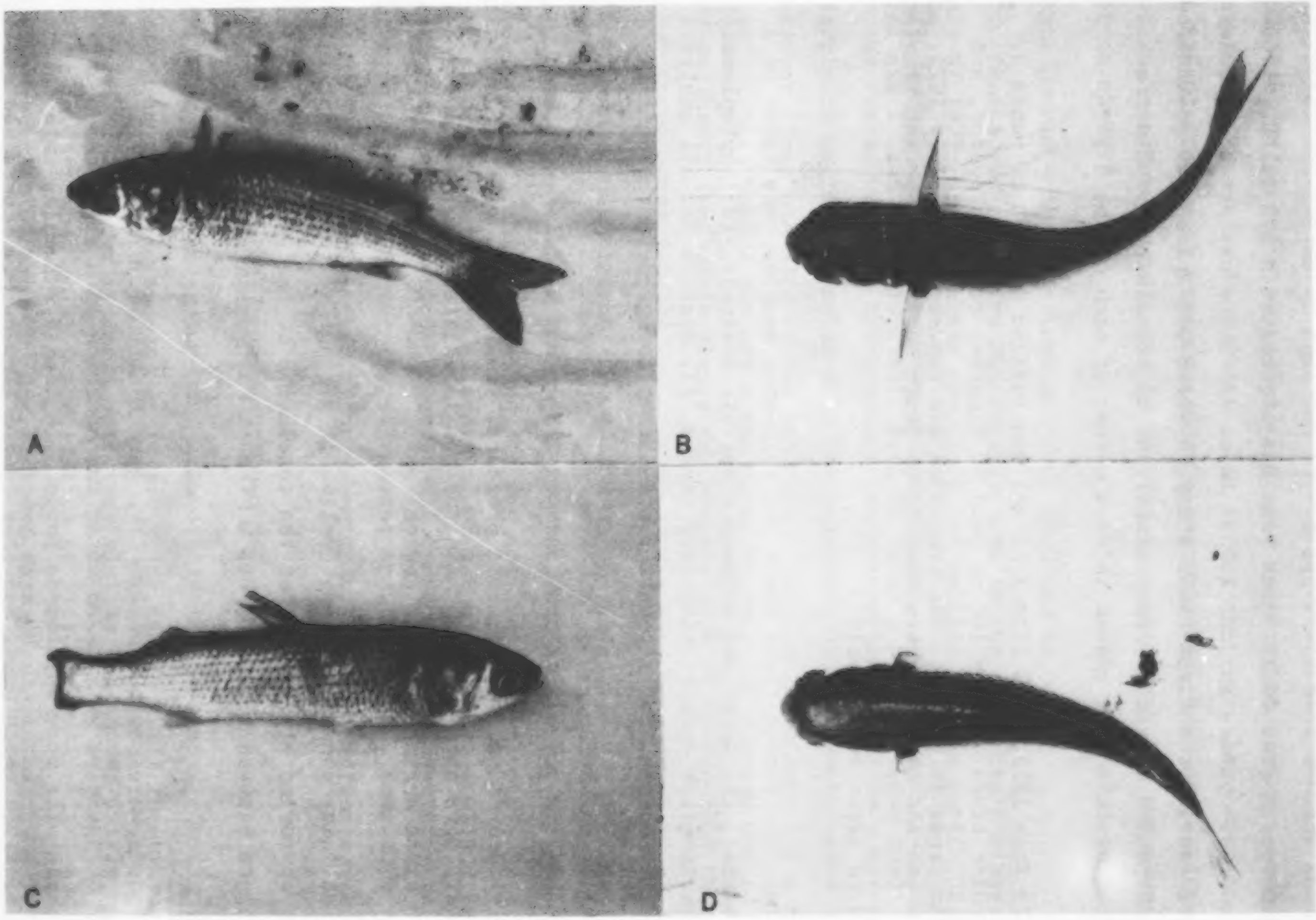

Figure 6. Photographs of mullet with various degrees of fin erosion. A and B--normal, C and D--very heavily eroded fins.

Accordingly, all remaining mullet were removed from the ponds, the salinity of the ponds increased to 13-14 ppt by the addition of salts, and the tidal simulation system shut down. Each pond was stocked with 350 mullet, 1400 shrimp and 400 oysters in June 1975. In an effort to obviate the problems encountered during the first field study, oysters were placed in galvanized baskets suspended on stilts approximately 60 cm beneath the pond surface. One hundred oysters were placed in each corner of each of the 4 ponds.

On 25 July 1975, 11.3 liters of oil was spilled in each of ponds 3, 4, and 5; Saudi Arabian crude was spilled in pond 3, Nigerian crude in pond 4, and Empire Mix crude in pond 5. The procedure was repeated on 27 July 1975, so that each test pond received a total of 22.6 liters of oil. Pond 2 was left untreated to serve as control.

During this second field study the following samples were taken on a routine basis: (1) water and sediments for oil analysis, (2) phytoplankton and zooplankton for species diversity counts, (3) oysters for oil analysis and bacterial counts, and (4) mullet and shrimp for gross pathological examinations.

Environmental Data

Daily recordings of barometric pressure, temperature, relative humidity and rainfall were obtained from NASA for the immediate area. No unusual events occurred during the test period.

The salinity showed a steady decline in all ponds from 13-14 ppt initially to approximately 9.5 ppt at the conclusion of the study. The dissolved oxygen ranged between 6 and 9 mg/1.

Fate Studies

The ponds were sampled on June 24, July 29 and October 6, 1975. The last 2 samples were taken after the addition of a small amount of various crude oils were spilled on Ponds 3, 4, and 5. Samples were taken from the middle of each pond, and only Pond 5 (Empire Mix) contained oil in the samples.

Control Pond (Pond 2)-

This was originally a control pond and was retained as a control pond for the second study. The aliphatic hydrocarbon distribution was unchanged throughout this period, but it was apparent that large amounts of the planted marsh grasses around the pond's edge had increased the natural biogenic organic carbon load in this pond over the two-year period. [221]

Pond Treated with Saudi Arabian Crude Oil (Pond 3)-

Pond 3 was an Empire Mix treated pond in the first field study. In July of 1975, Saudi Arabian crude oil was spilled in this pond. Chemical analysis one year after the first spill indicated that the oil had degraded to the degree that oil hydrocarbons (as determined by GC) were not recognizable in sediment samples with the exception of very small areas of sediment along one edge of the pond which apparently accumulated a heavier load of oil. Pond 3 has had a much greater input of organic matter into the sediments from marsh plants and grasses growing in the pond than the other three ponds. [222]

Pond Treated with Nigerian Crude Oil (Pond 4)-

Pond 4 was also an Empire Mix treated pond in the first field study. In July of 1975, Nigerian crude oil was spilled in this pond. During the year after the initial spill, several of the hydrocarbon analyses indicated fairly high concentrations of crude oil hydrocarbons. However, the oil was not evenly dispersed over the sediments; and, therefore, some core analyses did not indicate oil. Thus, assessment of oil in the sediments was dependent on sampling area. [223]

Pond Treated with Empire Mix Crude Oil (Pond 5)-

Pond 5 was a control pond in the original pond study. Empire Mix was spilled in this pond in July of 1975. Prior to the spill the hydrocarbon analyses of the pond sediment were very similar and showed little change either in distribution or concentration of hydrocarbons. After the spill the hydrocarbon analysis of the sediment changed drastically and indicated oil pollution even in the sample taken from the middle of the pond. Possibly more mixing occurred in this pond, causing some of the oil to reach the bottom sediments in the middle of the pond. On the other hand, there was more grass in the middle of the other ponds which might have adsorbed any oil coming in contact with it, reducing the amount of oil eventually reaching the sediments. [224]

Phytoplankton/Zooplankton

Again, as in the first chronic study, no quantitative differences were seen between the phytoplankton populations in the control pond and the oil-treated ponds. The apparent lack of effect of Empire Mix, Saudi Arabian and Nigerian on the phytoplankton populations in the respective ponds is in agreement with laboratory studies which predict little or no reduction in cell growth at the concentrations of oil found in the experimental ponds. The changes seen in the phytoplankton of the ponds during the study reflect seasonal and environmental changes rather than changes due to the acute or chronic effects of the crude oils. [225-228]

Prior to the second pilot-plant study (July 1975) zooplankton samples indicated a shift to a *Brachionus* (rotifer) population in all ponds. In June of 1974 (pre-first pilot-plant study) the population consisted of a near equal mixture of *Brachionus* and *Acartia tonsa* (copepod), both of which were present in relative low levels (<10 organism/l). However, by June of 1975 *Brachionus* had greatly increased in numbers (>100 organisms/l - control; 50 organisms/l - oil-treated) while the *Acartia* populations remained at about the same level as the summer of 1974 (<10 organism/l - all ponds). [225-228]

Immediately after the July 1975 oil spill, the *Acartia* populations declined sharply in the Saudi Arabian, Nigerian and Empire Mix treated ponds while remaining stable in the control pond. By late August (4-5 wks), the copepods in the oil-treated ponds had recovered to pre-spill levels. The *Brachionus* population remained stable in the control, Saudi Arabian and Nigerian-treated ponds while it declined in the Empire Mix-treated pond immediately following the spill and then recovered by late August. [225-228]

The zooplankton populations (*Acartia* and *Brachionus*) in all 4 ponds began a gradual decline about the first of September. This decline coincided with a heavy *Anacystis* (blue-green) bloom which occurred at that time. This factor prevented a further assessment of the impact of the test oils on the zooplankton populations. [225-228]

Core Sampling-

Culture procedures were modified for the second oil spill. One core sample was taken from each of the 4 ponds and mud taken 8 cm from the top of the core. This mud was used to inoculate agar media. Three kinds of media were used: BBM, Heinle 20 o/oo, and Heinle's Media at 3 o/oo. Liquid media were not used for counting.

Two days after the oil spill, a decline was noted in the algal populations in all the oiled ponds. Four days after the spill, all 3 oiled ponds showed an increase in algae. This was the only instance of a simultaneous change recorded in different ponds. Six days after the spill, the random patterns of growth and decline again appeared on the graphs, indicating the benthic population had returned to normal. [229-232]

In all, 224 liquid cultures and 1028 agar cultures were counted. Most of the algae seen in Heinle's Media were diatoms. BBM showed a wide range of genera throughout the experiment.

Microorganisms

An attempt was made during the second field study to relate changes in the microbial population in the oysters at various post-spill times to oil uptake. During the study period a total of 188 oysters were sampled from each pond. Plate counts and oil analyses were carried out for each individual oyster. No relationship was found between microbial plate counts and oil uptake by the oysters. The results may have been complicated by the high organic content in the ponds which apparently had a greater influence on the microflora than did the oil. These findings do not negate the possibility of a relationship between the two factors in the natural environment which is not generally as rich in organic matter as the oil-treated ponds were during the study period. [233-236]

Primary Productivity of Phytoplankton Community

The primary productivity of the phytoplankton community in the 4 ponds was determined by the carbon-14 technique described by Lind and Campbell (1969) and Vollenweider (1974). A total of 2 pre-spill and 6 post-spill samples were taken and measured for phytoplankton primary productivity.

Pre-spill measurements showed that Ponds 2 and 5 (control ponds in first study) had slightly lower phytoplankton production than Ponds 3 and 4 (ponds treated with Empire Mix crude oil in first study). Five days after the first respill, primary production in all 4 ponds was comparable. During subsequent post-spill measurements, primary production persisted to be generally higher in Ponds 3 (Nigerian) and 4 (Saudi Arabian) than in Ponds 2 (control) and 5 (Empire Mix). In addition, emulsified Empire Mix crude oil (10 and 100 mg/l) reduced phytoplankton growth under laboratory conditions. [237-238]

These observations are reflected further in the total pigment concentrations which also were found to be lower in Ponds 2 and 5 during pre-spill measurements, comparable among the four ponds 5 days after the first post-spill, and persisted to be lower than Ponds 2 and 5 during subsequent post-spill samples. It appeared that oil stimulated the growth of phytoplankton when present at low levels. [239]

Marsh Grass

On the fifth day following the initiation of the second field study, Juncus and Scirpus robustus were harvested from the control, Empire Mix, Saudi Arabian and Nigerian ponds. Following routine processing, these plants were analyzed for their oil content. In general, oil uptake in Juncus was higher than in Scirpus, higher in dead tissues than in live tissues and higher in the bottom parts than in the top parts of the plants. Oil was detected in plants from all the oil ponds while none was detected in plants from the control pond. It appears that Juncus has a greater affinity for Empire Mix than for Saudi Arabian or Nigerian. [240]

Mullet - Pathology

The results of the second pond study were different in many ways from those in the first study. With mullet the most striking difference was the lack of visible disease in the second study. As in the first study, the oil-exposed mullet were observed to be much more sensitive to handling and holding procedures than the controls; however, they did not develop fin rot. In the first study there was no impact on the growth of mullet. However, at the end of the second study there was a striking difference in the size of the mullet from the different ponds. When stocked, the mullet averaged 82 mm in length and averaged 15 g in weight. When harvested 9 1/2 mo later, the average lengths and weights were: (1) control mullet-151 mm; 65 g (2) Nigerian-treated mullet-129 mm; 35 g (3) Saudi Arabian-treated mullet-124 mm; 42 g (4) Empire Mix-treated mullet-150 mm; 64 g. From the data, it is obvious that the growth of the Nigerian- and Saudi Arabian-treated fish was adversely affected, while the growth of the Empire Mix-treated mullet was almost identical to the growth of the control mullet. This reduction in growth of the mullet and a similar reduction in the growth of oysters (see below), seems significant in the light of the uniformity of food supplies in all ponds.

The histological examination of the mullet samples taken at the end of the second study produced the following observations: (1) Mullet livers from all 3 oil-treated ponds had enlarged hepatocytes and indistinct cord structures. These conditions were similar to, but less severe than, those reported in the first study and were more localized. (2) The incidence of severely clubbed and fused gill filaments observed in the first study was not observed in samples from the Empire Mix- nor Saudi Arabian-treated ponds but was observed to a much lesser extent in samples from the Nigerian-treated pond.

When comparing the chronic effects observed in the first and second pilot-plant studies, it should be noted that the first study was terminated after 11 mo while the second lasted for nearly 9 1/2 mo.

Shrimp - Pathology

During the second field study in which 3 oils were spilled in separate ponds, an unusual condition was observed which was not previously encountered during this study nor cited in the literature. Oil was spilled in mid-July 1975. During the October sampling period, shrimp were obtained from the Empire Mix pond which had whitish spots at various places on their eyes. This condition has been termed the "white eye syndrome". Some had very small spots on the ventral side towards the posterior edge of the eye which were hardly visible, while others had large spots covering nearly half of their eyes (Figure 7). In sectioned material, the crystalline cones and the structures associated with the ommatidia (pigment cells, retinula cells, rhabdomes, etc.) were eroded away. Some brownish material, probably the remains of the pigment material which normally surrounds the ommatidia, could be seen in the lesions (Figure 7). These diseased areas were clearly non-functional. Usually both eyes were affected but not always to the same degree. Another sample was taken the first of November to make certain this was a commonly occurring problem. Significantly, nearly all of the shrimp from the Empire Mix pond had the "white eye syndrome" (18 out of 19) while only 6 out of 13 in the Saudi Arabian pond, and 7 out of 15 in the Nigerian pond had the "white eye syndrome". The pathology associated with the syndrome was much more severe in shrimp from the Empire Mix-treated ponds than from the Nigerian and Saudi Arabian ponds. Shrimp from the Nigerian and Saudi Arabian ponds often had only one or two very small spots on one eye while those from the Empire Mix pond almost always had large and small spots on both eyes. A total of 24 shrimp were obtained from the control ponds and all were normal. Additional samples could not be obtained because the weather turned cold and the shrimp buried in the mud. No additional samples were obtained from any of the ponds due to cold weather. Apparently the shrimp were unable to survive the cold winter, as none were obtained in the early spring nor during the final harvest.

Oysters - Pathology

While the experimental design of this portion of the study was constructed primarily to determine if there existed a relationship between the

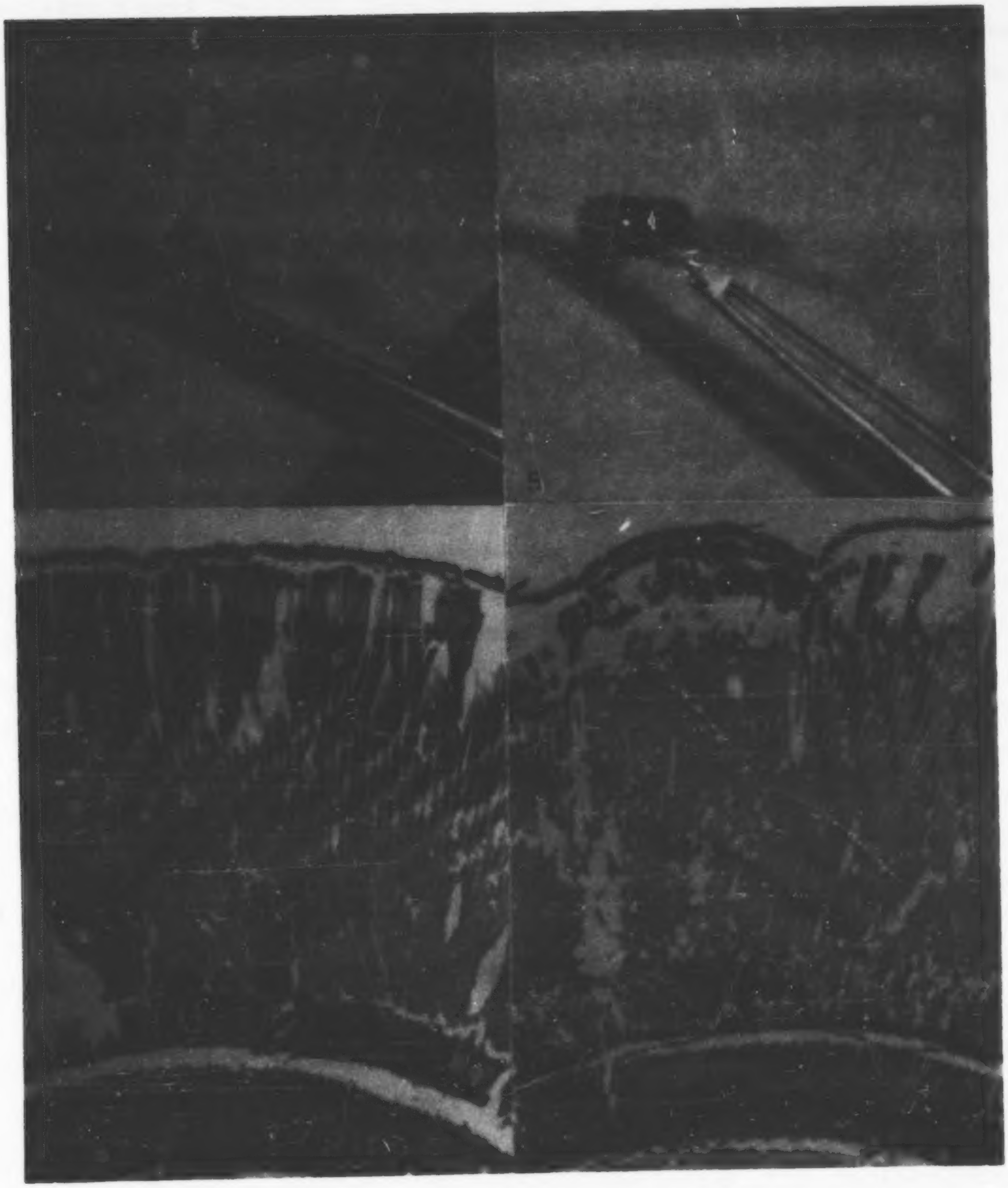

Figure 7. Photographs of whole and sectioned shrimp eyes. A--normal eye; B--eye with white spot; C--photomicrograph of a normal section of eye; D--photomicrograph with necrotic area visible just under corneal layer.

resident microflora and oil uptake by the oysters, the observation was made during the third and fourth sampling periods that the oysters from the Saudi Arabian- and Nigerian-treated ponds were in poorer health than those from the Control and Empire Mix-treated ponds. Based on this observation and on the lack of positive data from the microbial assays, routine sampling was suspended and the remaining oysters left undisturbed for the duration of the study. Unfortunately, by the time this decision was made a large number of the oysters in each pond (180 out of 400/pond) had been removed. Nevertheless, there was a distinct difference in the overall state of health and in the percent mortality of the surviving oysters in the various ponds at the time of harvest (9 1/2 mo exposure).

The oysters from the control pond were in very good condition. They were generally fat, had good color and 2 to 5 mm of new shell growth. The oysters in the Empire Mix pond did not appear as healthy as those in the control pond but were still in good condition; they had less color and were slightly watery. There had been some new shell growth, but in all cases it was less than 2 mm. The surviving oysters in the Nigerian and Saudi Arabian ponds were in very poor condition. They were clear, watery and appeared emaciated. Histologically, they resembled recently spawned oysters. One unusual histological alteration was the occurrence of fibrinoid degeneration of the connective tissue of the mouth and food groove of most surviving Nigerian-exposed oysters and a few Empire Mix-exposed oysters. [241]

If all oysters stocked (400/pond) are considered, the percent mortalities for the oysters stocked in each pond were: control, 23%; Saudi Arabian-treated, 54%; Nigerian-treated (50%); Empire Mix-treated, 31%. If those oysters removed for microbial analysis are not considered (180/pond), the percent mortalities would be: control, 42%; Saudi Arabian-treated, 85%; Nigerian-treated, 91%; Empire Mix-treated, 58%. In either case the percent mortalities for oysters in the Saudi Arabian- and Nigerian-treated ponds were much higher than those for the control or Empire Mix-treated ponds. If the mortality data is considered in conjunction with the observations made on the state of health of the surviving oysters, it must be concluded that the chronic exposure to low levels of Saudi Arabian and Nigerian crudes had an adverse effect on the state of health of the oysters. These observations seem even more significant when it is considered that the oysters were suspended in cages and consequently were only subject to impact by substances obtained from the water column directly or via the food chain.

SECTION VI

SUMMARY

INTRODUCTION

The format to be followed in the SUMMARY will parallel the RESULTS SECTION in that the Laboratory, Tidal-Pond and Pilot-plant studies will be summarized separately. A discussion of the merits of the pilot-plant system and an interpretation of the results will be presented in the DISCUSSION SECTION.

LABORATORY RESULTS

All laboratory experiments were carried out under controlled conditions with the exception of some of the marsh grass tests which were conducted in a natural estuarine environment. The following results in regard to the fate and effect of crude oil in the aquatic environment of the Gulf Coast Region have been obtained.

1. Empire Mix crude oil remains in the water column for only a short period of time during which some of it migrates into the sediments. Further, the degradation in, or disappearance from, the sediments is a slow process which is influenced by the oxygen content, by the presence of other sources of organic matter and by the availability of essential nutrients (phosphorus and nitrogen sources). Aromatic compounds are considerably more persistent than aliphatic compounds.

2. Phytoplankton species found in the estuarine environment vary in their susceptibility to crude oils with EC_{50} values ranging from 5.6 mg/l for Nigerian crude oil (tested against Skeletonema costatum) to greater than 58.0 mg/l for Empire Mix crude oil (tested against Carteria chiuu).

3. In general the zooplankton species found in the Gulf Coast environment are more susceptible to crude oil than are the phytoplankton with TL_m values for Acartia tonsa ranging from 0.55 mg/l for Nigerian to 1.62 mg/l for Venezuelan.

4. From the marsh grass studies it is estimated that (A) a single oil spill with Empire Mix at a dosage of 250-1500 ml of oil/m^2 of marsh will reduce marsh plant productivity by 400-900 g/m^2. (B) Repeated spills with Saudi Arabian oil at 600-6000 ml of oil/m^2 of marsh at the rate of 600 ml/mo will reduce marsh plant productivity by 500-900 g/m^2. (C) Dead marsh plants (Juncus) contaminated (by soaking) with Empire Mix and Saudi Arabian crude oils decomposed 40 to 50% slower than the control (unoiled) after 323 days in the field; therefore, natural degradation of marsh plants to detritus is hindered by oil contamination. (D) Oiled dead plants and the detrital material which is eventually formed from them serves as a mechanism for the long term entry of oil and/or oil degradation products into the marsh-estuarine food chain.

5. Accumulation of crude oil by marsh grass and phytoplankton was shown to occur, but the data suggest that this is more a physical than a biological phenomenon.

6. Crude oil was not highly toxic to mullet in 96-hr bioassays. However, the 5 crude oils varied in their toxicity to mullet with Nigerian the most toxic (TL_m less than 200 mg/l; 100% mortality at 200 mg/l), Saudi Arabian the next most toxic (TL_m less than 350 mg/l; 100% mortality at 350 mg/l), Iranian and Venezuelan intermediate (TL_m between 400-800 mg/l) and Empire Mix the least toxic (TL_m 800 mg/l). At laboratory treatment levels of 25 and 75 mg/l both Empire Mix and Saudi Arabian caused severe fin rot outbreaks. Fish with some fin erosion were generally observable beginning at about 8-10 days after treatment, with 100% mortality occurring after 18-25 days. A Vibrio was tentatively identified as the causative agent. Behaviorally, changes were restricted to hyperactivity which was more pronounced following exposure to the more toxic oils. Some oil uptake by the tissues was observed, and changes in fatty acids were noted although no

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histological changes were evident. Changes in enzymes associated with stress and/or detoxication were observed following exposure to crude oil. In addition, certain microsomal oxidases were induced in livers following short-term exposures to Empire Mix and Saudi Arabian.

7. Bioassay tests with shrimp using different crude oils showed that Nigerian was the most toxic (TL_m 10 mg/l), Empire Mix, Saudi Arabian, and Iranian intermediate (TL_m 15-25 mg/l) and Venezuelan the least toxic (TL_m 35-45 mg/l). Behaviorally, the most significant response of shrimp to crude oil was their tendency to "spiral". In nature, this "spiralling" would most likely result in predation thus eliminating the possibility of recovery. Enzymatically, there were no significant changes in the hepatopancreas following exposure of shrimp to Empire Mix. However, treatment with Saudi Arabian resulted in great alterations in the activities of certain enzymes of the hepatopancreas. Uptake of oil by the shrimp tissue was highest during the first 24 hrs of exposure. The fatty acid profiles changed after exposure of the shrimp to oil but tended to normalize with time. Histologically, abnormalities were noted in the gills, mandibles, gastric mill and inner lining of the carapace of shrimp exposed to Empire Mix.

8. The general response of oysters to the addition of crude oil at the levels tested was to close and cease pumping. This response precluded the acquisition of data on lethality and complicated the interpretation of other data. No histological changes or changes in fatty acid composition could be attributed to exposure to oil. Some alterations of enzyme activity were noted, which could be a reflection of the long periods of closing.

TIDAL POND STUDY

Two naturally stocked ponds (one control and one treated) adjacent to Davis Bayou, which adjoins Gulf Coast Research Laboratory in Ocean Springs, Mississippi, were chosen as sites for the tidal-pond study. A levee was built up with sandbags to a height above the high tide level thus entrapping a natural population of organisms. The ponds were monitored prior to and following the oil spill (250 mg/l Empire Mix crude) for a period of 18 mo.

This tidal-pond study produced the following results concerning the fate and effect of crude oil in the aquatic environment of the Gulf Coast Region.

1. High concentrations (32.5 mg/l) of oil in the water column were found 1 day after the spill followed by entrainment of the hydrocarbons into the sediment. On the basis of aliphatic components, the crude oil was still evident in the sediments 12 mo later. After this time, the contribution of biogenic aliphatics appeared to mask the contribution of crude oil aliphatics, but the top portion of the sediments still retained an increased aromatic content.

2. Oil-sensitive phytoplankton were killed, as evidence by a 43-65% reduction in primary productivity within 16 days. The primary productivity returned to normal in 2 mo and there was an increased abundance and diversity of phytoplankton in the oil pond (as compared to the control pond) 1 yr later.

3. Oil-sensitive zooplankton were killed initially. As the oil dissipated, there was a rapid increase in zooplankters, and after 6 mo the population was similar to that in the control pond in both abundance and diversity and exceeded those in the control pond in abundance after 12 mo.

4. Oil uptake by and decreased productivity of marsh grass was observed.

5. Under conditions of the test, data on mortalities of the macrofaunal population were impossible, but predation of erratically swimming menhaden by ladyfish was observed.

FIRST PILOT-PLANT ECOSYSTEM STUDY

The major thrust of this contract was to construct and employ a pilot-plant ecosystem to study the long-term effects of a small quantity of crude oil on the ecosystem. Physically the system consisted of two sets of ponds (46 x 46 x 2.5 m) connected by means of a tidal simulation system which lowered the water level in one pond by 46 cm every 12 hrs then reversed. Salinity was established and maintained between 6-12 ppt through the addition of seawater and salts. Marsh grass planted along three sides of each pond was intended to contribute nutrients for the shrimp, mullet and oysters in the pond.

Overall performance of the system was excellent. The marsh grass flourished, and the phytoplankton and zooplankton populations that developed were representative of the estuarine areas of the Gulf Coast. The mullet (in the control ponds) appeared healthy, and normal growth was obtained. The shrimp exhibited excellent growth, survived the first winter which was mild but failed to survive the second winter. Oysters did extremely well for the first yr and displayed growth rates in excess of those normally found in nature. The gradual accumulation of silt resulted in the loss of all oysters during the experiment.

The emphasis in this first study was directed toward (1) assessing the value of laboratory data when tested on a pilot-plant basis under conditions resembling the environment and (2) ascertaining if studies on a pilot-plant basis would yield results not observed in the laboratory.

The first test using the system involved two additions of 11.3 liters of Empire Mix crude oil to each test pond, 48 hrs apart. The following information was obtained from this 11 mo study.

1. The results confirmed the laboratory findings in respect to the long residence time of the Empire Mix crude oil in the sediments and the persistence of the aromatic compounds in the system.

2. The level of oil employed in this study did not have a measurable effect on the number of bacteria, yeasts, fungi or actinomycetes. The only observed impact of oil on the population was the increase in hemolytic bacteria in the oil-treated ponds during the first 13 days following the spill.

3. The response of the phytoplankton and zooplankton to Empire Mix crude oil was in agreement with the laboratory data, namely that (1) less

than 10 mg/l stimulated the phytoplankton, (2) its effects varied depending on the phytoplankters involved; for example Oscillatoria, a cyanophycophytan, accounted for the majority of the observed phytoplankton increase while the chlorophycophytan and chrysophycophytan species present increased very little following the oil spill, (3) low levels (less than 10 mg/l) caused a drastic reduction in specific zooplankters (Acartia; Brachionus), and (4) the zooplankters recovered to pre-spill levels within a short period of time (10-15 days). There was no measurable change in sedimentary algal populations as a result of exposure to Empire Mix crude oil. It should be noted, however, that these algal populations were distributed in a checkerboard fashion rather than distributed evenly which made the interpretation of data difficult.

4. The oysters grew exceptionally well during the early stages of the study but were subsequently lost because of silting; thus, no specimens were available after 4 mo. The oysters demonstrated an uptake of low levels of oil followed by depuration. Although no statistically significant differences in the level of enzymes were found, some of the changes suggested that certain biochemical and physiological processes may be affected for several mo after the oil spill. Data on fatty acid content was in agreement with a stress response. No abnormal pathological conditions were noted but general poor health of the oysters was observed.

5. Since very few of the shrimp which had been placed in the ponds 8 mo prior to the spill survived the winter, the availability of specimens was decreased. Thirty-six per cent of the pooled hepatopancreas samples after the oil spill had an elevated aromatic content; this suggested that the shrimp obtained oil from the sediments or their food rather than from the water column. The fatty acids in oil-treated shrimp showed a trend toward chain elongation and unsaturation. This trend could be attributed to a stress response. Changes in β-glucuronidase may be an indication of a long-term effect on some physiological process in the shrimp. No pathological abnormalities attributable to oil were observed.

6. Oil uptake in mullet varied according to the tissue sampled. No significant amounts of oil were found in gill samples at any time during the study. All brain samples contained low levels of oil. Liver samples taken 11 days after the spill had detectable but low levels of oil while those samples taken shortly thereafter had no detectable oil. However, samples taken about 5 mo after the spill as well as all samples taken thereafter contained "oil." It should be noted that the chromatographic characteristics of those aromatic hydrocarbons peaks in these liver tissue samples differed from those found in the Empire Mix crude oil itself. There was a general shift to shorter retention times for the predominant peaks in the samples from the oil-treated ponds thus indicating an increase in polarity of those compounds present. In general the enzyme data reflected a stress response or the general poor health of the oil-exposed fish. Fatty acid analyses reflected a pattern characteristic of a stress response. Histologically, chronic exposure (9-10 mo) resulted in severe hyperplasia, clubbing and fusion of the gill filament accompanied by a large increase in the number of mucoidal cells. The hepatocytes of the oil-exposed fish were enlarged causing a decrease in the sinusoidal spaces and a distortion of the normal appearance of the hepatic cords. In addition, there appeared to be a general loss of glycogen from the cells with an increase in fat vacuoles. These changes were subtle in the earlier stages of the study (4-6 mo) but were very pronounced and were present in a majority of the samples (20/pond) obtained from the treated ponds when the test was terminated (11 mo). These conditions were rarely observed in control fish and when they were, they were localized as opposed to very general in the liver tissues of treated fish.

7. By far the most dramatic finding during the first pilot-plant study was the development of fin rot in essentially 100% of the fish in the oil-treated ponds.

SECOND PILOT-PLANT ECOSYSTEM STUDY

The second test using the pilot-plant ecosystem involved monitoring the effect(s) of low levels of Saudi Arabian, Nigerian and Empire Mix without the influence of tidal simulation. A majority of the mullet were removed from the ponds after the first test and the ponds were restocked by the addition of mullet, shrimp and oysters to each pond. For this study the oysters were placed in wire baskets suspended in the ponds.

Since the emphasis in this test was to determine if the different crudes elicited different responses, 22.6 liters of Empire Mix was added to one pond, 22.6 liters of Nigerian added to a second pond, and 22.6 liters of Saudi Arabian added to a third pond. The fourth pond served as a control.

This second static pilot-plant ecosystem study yielded the following significant results.

1. The persistence of oil in the sediments was confirmed. There was a more even distribution of petroleum hydrocarbons in the sediments probably reflecting the discontinuation of the use of the tidal simulation system.

2. The response of phytoplankters to the 3 oils tested was essentially the same as in the previous laboratory and pilot-plant tests indicating a high tolerance to crude oil.

3. The response of zooplankters varied depending upon which oil was employed: Empire Mix caused a significant reduction in the Brachionus (rotifer) and Acartia (copepod) populations. This is in agreement with the first pilot-plant study. However, the Brachionus population was unaffected by Nigerian and Saudi Arabian while the Acartia was drastically reduced by both oils. All changes in the zooplankton populations were short-term.

4. Marsh plant data generally agreed with the data from previous studies in that uptake was higher in roots than in shoots and higher in dead tissue than in live tissue. New observations made during this study were (1) oil uptake by Juncus was higher than by Scirpus and (2) Juncus had a greater affinity for Empire Mix than for Nigerian or Saudi Arabian.

5. Data from the second field study indicated a difference in the impact of the 3 test oils on the growth of mullet. When stocked the

mullet averaged 82 mm in length and 15 g in weight. When harvested 9 1/2 mo later the average lengths and weights were: (1) control mullet: 151; 65 g , (2) Empire Mix-treated mullet: 150 mm; 64 g , (3) Saudi Arabian-treated mullet: 124 mm; 42 g , and (4) Nigerian-treated mullet: 129 mm; 35 g. Histologically, the changes observed in the hepatocyte cells of the mullet from all 3 oil-treated ponds were similar by less severe than those observed during the first pilot-plant study. However, of the 3 crudes tested Nigerian was the only one which caused the gill pathology observed in the first study. Here too, the pathology was less severe. The outbreak of fin rot observed in the first pilot-plant study did not occur during this second study. However, there was an impact on growth as indicated by the significant reduction in the lengths and weights of the mullet in the Nigerian- and Saudi Arabian-treated ponds as compared to the Empire Mix-treated and control ponds.

6. The suspension of the oysters in wire baskets eliminated the problem of high mortalities associated with silting which was encountered in the first pilot-plant study. The original intent of this phase of the study was to determine if there was a correlation between microbial counts and oil uptake in the oysters. While no correlation was found, the following observations were made on those oysters remaining in the ponds at the termination of the study: (1) The control oysters were fat, had good color and 2.5 mm of new shell growth. There was a total mortality of 97 out of 400; (2) The oysters in the Empire Mix-treated pond were not as fat, had less color and were slightly watery. There was a total mortality of 127 out of 400; and (3) The oysters in the Nigerian and Saudi Arabian ponds were emaciated, lacked any color and were very watery. The total mortalities were 201 and 186, respectively.

7. A heretofore unreported disease, designated as the "white eye syndrome" was observed in the shrimp exposed to crude oil. The prevalence of the syndrome varied with the oil employed and ranged from 95% for Empire Mix, to 46% for the Saudi Arabian and Nigerian. Grossly, the diseased areas appeared as white opaque spots varying from very small to very large (covering up to 1/2 of the involved eye). Histologically, the crystalline

cones and the structures associated with the ommatidia were completely destroyed. No other damage to the eye was observed.

SECTION VII

DISCUSSION

The pollution of the marine environment by petroleum products has been documented since 1754 (Nelson-Smith, 1973) but has not been of major concern because of man's belief that the vastness of the oceans and seas is such that they have an unlimited ability to absorb pollutants. Early studies revolved around the impact of oils on specific economically important species such as the oyster (Galtstaff, et al., 1973; Mackin and Sparks, 1961; Mackin and Hopkins, 1961) and often were initiated in response to charges that crude oils were adversely affecting the industries related to these organisms. While some laboratory data indicated possible deleterious effects, the observations could not be confirmed in the field and were generally considered questionable. Then in 1967 the Torrey Canyon incident incited public outrage due to its direct impact on man's activities at a time when the general public was sensitized to environmental considerations. This outrage resulted in voluminous survey type studies on the effects of this and other major oil spills which followed. Most of these studies of necessity involved surveys of the living and dead organisms following the spills with follow-up surveys to determine the time required for recolonization. They were complicated by the use of emulsifiers which were sometimes more deleterious than the oils themselves. In general, these studies concluded that there was a potential for severe short-term impact of oil especially on intertidal organisms and aquatic birds. However, the marine ecosystems were believed to recover in a short time with no long-term impact. One exception to this general trend was the studies surrounding the West Falmouth spill (Blumer, et al., 1970; Blumer, et al., 1971; Blumer and Sass, 1972) which indicated that No. 2 fuel oil not only was acutely toxic to a wide range of organisms but remained in the system essentially unaltered for a considerable length of time. Data obtained in these and other studies surrounding this spill indicated that the environment was altered by the presence of the fuel oil for a number of years.

These seemingly opposing views about the long-term effects of oil precipitated much needed scientific and governmental interest in clarifying the long-term effects of oil under natural or seminatural conditions. The tremendous variability in the marine environment and the complex nature of crude oils and refined petroleum products resulted in many arguments in the scientific community as to the best approach to utilize in attacking the problem.

The need for complementing laboratory studies and field investigations with pilot-plant ecosystem studies has become evident. Basically, the system should include a complete but limited food chain which would operate without additional manipulation. The system would be employed to (1) verify laboratory findings on a more realistic scale, (2) establish effects not observed in laboratory studies and (3) study long-term chronic effects in a system simulating the natural environment. One of the major questions regarding the establishment of a large scale pilot-plant ecosystem is how well it simulates the natural environment.

In the present investigation, pre-spill data on the pilot-plant ecosystem and the performance of the control ecosystem indicate that not only did the system perform adequately, but it also simulated the ecosystem of the estuarine area of the Gulf Coast. The ecosystem ponds employed in this investigation were filled with natural seawater (28 o/oo salinity) diluted with freshwater to a salinity of 14 o/oo. Decreases in salinity occurred as a result of dilution with rainwater but was maintained at 6.0 - 14.0 o/oo through the addition of salts or natural seawater. While the salinity in the estuaries of the Gulf Coast generally range from 0.0 to 24.0 o/oo, the narrower range employed in the ponds was felt to be advantageous to the present investigation. The planktonic community was typical of the estuaries and exhibited natural fluctuations in response to changes in salinity and season.

Although no sediments were added to the ponds, a rich organic sediment developed within the first 15-18 mo. The contribution of organic detritus from the marsh grass, phytoplankton and other sources to the sediment was clearly evident from the chemical analyses. The bacterial flora of the water column and sediments was typical of the Gulf Coast estuaries in numbers and diversity. The newly developed Tidal Simulation System functioned extremely well and was probably responsible for transporting the marsh grass detritus into the system.

The mullet, shrimp and oysters which were introduced into the system thrived and appeared to be in excellent health. In fact, during the first several mo in the system, the growth of the oysters exceeded that normally occurring in the estuaries. These findings are especially important in view of the fact that artificial feeding was limited to a small amount of feed added for "training" the fish and shrimp to congregate to facilitate sampling. Two major problems were encountered. First, the shrimp survived the first winter (unusually mild) but failed to survive the second winter. In studies spanning a year or more, shrimp would probably have to be reintroduced each spring. The second problem was concerned with the death of the oysters after they became buried in the sediments. This problem was overcome in the second study by placing the oysters in wire baskets suspended beneath the surface of the ponds. Not only did the oysters do well under these conditions, but histological examinations indicated that they underwent normal sexual development.

In essence, the artificially constructed pilot-plant ecosystems functioned extremely well throughout the course of the investigation and proved to be a valuable tool in assessing the effect of long-term, low-level oil exposure on an ecosystem basis. For example, the pilot-plant ecosystem studies confirmed the laboratory findings in regard to the effect of oil on the planktonic community, thus increasing confidence in extrapolating these results to the natural environment. These studies also verified the fact that zooplankters were the most oil-sensitive members of the ecosystem and further demonstrated that their recovery was rapid even in the absence of an exogenous source of new organisms. This recovery indicated not only that a sufficient number of organisms survived the spill, but also that the system itself had recovered to the point that it was no longer inhibitory or toxic to these forms of life. There was, however, a shift in population from the copepod Acartia to the rotifer Brachionus. Further, the studies confirmed the long residence time of oil in the sediments and clearly demonstrated the problems of acquiring representative samples and interpreting results of hydrocarbon analyses in the presence of other sources of hydrocarbons. The failure of the crude oil to cause shifts in the microbial population was probably a reflection of the small amount of oil employed and the presence of a large quantity of other organic compounds. These results indicate that the fate of oil in the environment will be influenced by the quantity of biologically available organics as well as the concentration of the oil.

While histological changes were observed in oil-exposed shrimp in the laboratory studies, none were observed in the specimens in the pilot-plant ecosystem. This was probably a reflection of the problem of obtaining representative samples of the shrimp from the ponds.

The pilot-plant ecosystem did not contain predators for the mullet, shrimp or oysters; therefore, the predation which was observed in the tidal pond studies or which may well be observed in any field study, did not occur in the pilot-plant system. The inclusion of predators, such as lady fish, speckled trout, etc., would have afforded the opportunity to make these observations in the pilot-plant system. However, the exclusion of predators from the system resulted in the finding of two heretofore unreported effects of oil pollution: namely, fin rot in fish and "white eye syndrome" in shrimp. Both of these effects would not be observed in field investigations, since the obvious impairments of the affected organisms would probably have resulted in their demise through predation. The exact cause of these two conditions has not been established but the results clearly indicate that they are either directly or indirectly caused by oil.

Use of the pilot-plant ecosystem also led to several significant findings relative to the long-term effect of oil pollution. Histological changes in the liver of mullet began to appear 4-6 mo after the oil spills. Since these changes were not observed in the livers of control fish it was concluded that they were caused by oil. The question as to whether these changes were a result of uptake of oil immediately after the spill or whether they were brought about by the continual exposure to oil or oil degradation products is not known. Aromatic hydrocarbon profiles from the livers of mullet 5 or more mo after the oil spill were suggestive of polar (oxidized) metabolites of oil components. These polar materials could be the result of either uptake and storage of microbial degradation products or oxidation of oil components by the fish themselves. The latter option is a distinct, although unconfirmed, possibility based on laboratory data indicating the presence of certain microsomal hydroxylation enzymes in mullet livers, which are inducible by crude oil.

The exact mechanism responsible for the decreased growth of oil-exposed mullet during the second pilot-plant ecosystem study is not known. The increased mortality of the oysters during the second pilot-plant study did not occur until several mo after the oil spill. Since these organisms are sessile and were suspended in the water column only materials suspended and/or dissolved in the water column could have been responsible. It is possible that any or all of these effects could have been a result of a dietary deficiency brought about by the impact of the oil on lower members of the food chain or by interference with the oysters' ability to utilize their food, although no evidence to this effect was obtained during the study. A clearer understanding of the cause and effect relationship of oil to these observations is urgently needed. For example, if these findings are the result of the uptake of oil immediately after the spill, the damage to these members of the ecosystem would be limited to those organisms in the immediate area of the spill. On the other hand, if these problems are a result of oil in the sediments or in the detritus, the magnitude of damage to the ecosystem could be many times greater.

There has been a tendency to rank oils according to their toxicity on the basis of acute toxicity tests such as LD_{50} determinations. For example, the high sulfur crudes like Saudi Arabian are usually thought to be more toxic than the low sulfur crudes like Empire Mix. The present investigation tends to substantiate this opinion in most cases but was reversed in a few cases. The more volatile components of the crudes are considered to be more toxic than the less volatile constituents and the results in the present investigation tends to support this view. In summary, crude oils do vary in toxicity, and further organisms vary in their response to different crudes. It should also be emphasized that while Empire Mix crude oil would have to be considered the least toxic of the 5 crudes tested in this investigation from the standpoint of the short-term experiments, it did cause both short-term and chronic effects even when employed in low concentrations.

Not only does the specific type of crude oil make a difference in the results but also the environmental conditions under which the test is conducted influences the findings. For example, an outbreak of fin rot occurred in the first test but not the second test. At first this seems surprising since the same amount of oil was spilled in both studies. However, the fact that the second test was in a static, not a flowing test system, could well account for the difference. The static system by reducing the mixing allowed the mullet the opportunity to avoid the oil. In addition, the lack of mixing in the second study allowed the oil to remain on the surface longer, facilitating the volatilization of more of the lighter fractions of the oil. This would reduce the likelihood of physical or chemical actions of these components on the mullet. Also, the mullet in the second study were smaller (45-80 mm) than those used in the first study (130-170 mm). This size difference may account for behavioral differences observed in the mullet population in the ponds during the two study periods. During the first spill the larger mullet were constantly observed hanging at the surface (piping), generally swimming in and around the oil slick. Contrary to this, however, the mullet in the second study were rarely observed at the surface prior to or during the spill. This behavioral difference could have limited the contact of the smaller mullet with the oil slick. This contention was supported by the observation that exposure to Empire and Saudi Arabian crudes in aerated aquaria and vats (thus mixed) consistently caused fin rot in mullet in 8-10 days.

Another significant difference between the two chronic studies was the occurrence of the unusual "white eye syndrome" in shrimp only during the second study. No reason for this difference can be given. The static conditions seemed to have resulted in a more uniform distribution of oil in the sediments which increased the opportunity of the shrimp to contact the oil.

Finally it should be emphasized that overall the results of the pilot-plant ecosystem studies produced results which clearly indicated the debilitating effect of even a low level of oil on higher members of the estuarine food chain.

In fairness, it must be pointed out that these observations were made in a closed system and, whether or not the same kinds and magnitudes of effects would occur in nature, is obviously unknown. It would be fair, however, to predict that if a natural system is subjected to levels of oil, as employed in these experiments, whether or not as a single dose or a multi-dose situation, that similar effects could be anticipated.

SECTION VIII

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16. ABSTRACT

The purpose of this research investigation was to determine the fate and effect of crude oil in the aquatic environment of the coastal Gulf of Mèxico. The project was multi-disciplinary and multi-institutional in scope and involved both laboratory and field sized pilot-plant ecosystem studies. Emphasis was placed on the long-term, low-level chronic effects of oil pollution on the ecosystem. Of the five crudes employed in the investigation, Empire Mix crude was studied most intensively.

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